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(71) Applicant *(for all designated States except US)*: GENZYME CORPORATION [US/US]; One Mountain Road, Framingham, MA 01701 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: ROMANCZUK, Helen [US/US]; 3101 Windsor Ridge Drive, Westboro, MA 01581 (US). ARMENTANO, Donna [US/US]; 352 Brighton Street, Belmont, MA 02178 (US). O'RIORDAN, Catherine, R. [IE/US]; 70 Revers Street, Boston, MA 02114 (US).

(74) Agent: SEIDE, Rochelle, K.; Baker & Botts, LLP, 30 Rockefeller Plaza, New York, NY 10112-0228 (US).

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(57) Abstract

The present invention is directed to adenoviral vectors having modified capsid proteins which comprise heterologous ligands that improve and/or alter the infectious capability of the vector. Such ligands are capable of binding to target cells, and their inclusion into adenoviral vectors facilitates the binding and infectious properties of the vectors. In a preferred embodiment, the ligands are peptides, and the target cells are epithelial cells. The invention is also directed to novel heterologous ligands, to ligand–receptor complexes, and to compositions comprising the adenoviral vectors of the invention. Additional aspects of the invention include methods to use the adenoviral vectors of the invention to deliver transgenes to target cells.

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Description

Adenoviral Vectors With Modified Capsid Proteins

Introduction

The present invention is directed to adenoviral vectors having modified capsid proteins which comprise heterologous ligands that improve and/or alter the infectious capability of the vector. Such ligands are capable of binding to target cells, and their inclusion into adenoviral vectors facilitates the binding and infectious properties of the vectors. The invention is also directed to compositions comprising the adenoviral vectors of the invention and methods for the use of these adenoviral vectors to deliver transgenes to target cells.

Background of the Invention

Adenovirus (Ad) is a nuclear DNA virus with a genome size of about 36 kb, which has been well-characterized through studies in classical genetics and molecular biology. A detailed discussion of adenovirus is found in Shenk, T., "Adenoviridae and their Replication", and Horwitz, M.S., "Adenoviruses", Chapters 67 and 68, respectively, in Virology, B.N. Fields et al., eds., 2nd edition, Raven Press, Ltd., New York, 1996, and reference therein is found to numerous aspects of adenovirus pathology, epidemiology, structure, replication, genetics and classification.

In a simplified form, the adenoviral genome is classified into early (known as E1-E4) and late (known as L1-L5) transcriptional units, referring to the generation of two temporal classes of viral proteins. The demarcation between these events is viral DNA replication.

The human adenoviruses are divided into numerous serotypes (approximately 47, numbered accordingly and classified into 6 subgroups: A, B, C, D, E and F), based upon properties including hemagglutination of red blood cells, oncogenicity, DNA and protein compositions and relatedness, and antigenic relationships.

Recombinant adenoviruses have several advantages for use as gene transfer vectors, including tropism for both dividing and non-dividing cells, minimal pathogenic potential, ability to replicate to high titer for preparation of vector stocks, and the potential to carry large inserts (Berkner, K.L., Curr. Top. Micro. Immunol. 158:39-66, 1992; Jolly, D., Cancer Gene Therapy 1:51-64, 1994).

The cloning capacity of an adenovirus vector is proportional to the size of the adenovirus genome present in the vector. For example, a capacity of about 8 kb can be created from the deletion of certain regions of the virus genome dispensable for virus growth, e.g., E3, and the deletion of a genomic region such as E1 whose function may be restored in trans from 293 cells (Graham, F.L., J. Gen. Virol. 36:59-72, 1977) or A549 cells (Imler et al., Gene Therapy 3:75-84, 1996). Such E1-deleted vectors are rendered replication-defective, which is desirable for the engineering of adenoviruses for gene transfer. The upper limit of vector DNA capacity for optimal carrying capacity is about 105%-108% of the length of the wild-type genome. Further adenovirus genomic modifications are possible in vector design using cell lines which supply other viral gene products in trans, e.g., complementation of E2a (Zhou et al., J. Virol. 70:7030-7038, 1996), complementation of E4 (Krougliak et al., Hum. Gene Ther. 6:1575-1586, 1995; Wang et al., Gene Ther. 2:775-783, 1995), or complementation of protein IX (Caravokyri et al., J. Virol. 69:6627-6633, 1995; Krougliak et al., Hum. Gene Ther. 6:1575-1586, 1995). Maximal carrying capacity can be achieved using adenoviral vectors deleted for all viral coding sequences (allowed U.S. Patent Application No. 08/895,194; Kochanek et al., Proc. Natl. Acad. Sci. USA 93:5731-5736, 1996; Fisher et al., Virology 217:11-22, 1996).

Transgenes that have been expressed to date by adenoviral vectors include, *inter alia*, p53 (Wills et al., Human Gene Therapy 5:1079-188, 1994); dystrophin (Vincent et al., Nature Genetics 5:130-134, 1993; erythropoietin (Descamps et al., Human Gene Therapy 5:979-985, 1994; ornithine transcarbamylase (Stratford-Perricaudet et al., Human Gene Therapy 1:241-256, 1990; We et al., J. Biol. Chem. 271;3639-3646, 1996;); adenosine deaminase (Mitani et al., Human Gene Therapy 5:941-948, 1994); interleukin-2 (Haddada et al., Human Gene Therapy 4:703-711, 1993); and α1-antitrypsin (Jaffe et al., Nature Genetics 1:372-378, 1992); thrombopoietin (Ohwada et al., Blood

88:778-784, 1996); and cytosine deaminase (Ohwada et al., Hum. Gene Ther. 7:1567-1576, 1996).

The particular tropism of adenoviruses for cells of the respiratory tract has particular relevance to the use of adenovirus in gene therapy for cystic fibrosis (CF), which is the most common autosomal recessive disease in Caucasians. The disease is caused by the presence of one or more mutations in the gene that encodes a protein known as cystic fibrosis transmembrane conductance regulator (CFTR), and which regulates the movement of ions (and therefore fluid) across the cell membrane of epithelial cells, including lung epithelial cells. Abnormal ion transport in airway cells leads to abnormal mucous secretion, inflammmation and infection, tisssue damage, and eventually death. Mutations in the CFTR gene that disturb the cAMP-regulated Cl^- channel in airway epithelia result in pulmonary dysfunction (Zabner et al., Nature Genetics 6:75-83, 1994). Adenovirus vectors engineered to carry the CFTR gene have been developed (Rich et al., Human Gene Therapy 4:461-476, 1993) and studies have shown the ability of these vectors to deliver CFTR to nasal epithelia of CF patients (Zabner et al., Cell 75:207-216, 1993), the airway epithelia of cotton rats and primates (Zabner et al., Nature Genetics 6:75-83, 1994), and the respiratory epithelium of CF patients (Crystal et al., Nature Genetics 8:42-51, 1994). Recent studies have shown that administering an adenoviral vector containing a DNA encoding CFTR to airway epithelial cells of CF patients can restore a functioning chloride ion channel in the treated epithelial cells (Zabner et al., J. Clin. Invest. 97:1504-1511, 1996; U.S. Patent No. 5,670,488 issued September 23, 1997).

Adenoviruses are nonenveloped, regular icosahedrons (having 20 triangular surfaces and 12 vertices) that are about 65-80 nm in diameter. A protein called fiber projects from each of the vertices. The fiber protein is itself generally composed of 3 identical polypeptide chains, although the length thereof can vary from serotype to serotype. The protein coat (capsid) is composed of 252 subunits (capsomeres), of which 240 are hexons, and 12 are pentons. Each penton comprises a penton base, on the surface of the capsid, and a fiber protein projecting from the base. The Ad 2 penton base protein, for example, has been determined to be an 8 x 9 nm ring shaped complex composed of 5

identical protein subunits of 571 amino acids each. Adenovirus protein IX is also located on the surface of the viral capsid.

Current understanding of adenovirus-cell interactions suggests that adenovirus utilizes two cellular receptors to attach to and, then, infect a target cell. It has been suggested that the fiber protein of an infecting adenovirus first binds with high affinity to a cellular receptor; subsequently, the viral penton base interacts with cellular alpha-integrins, leading to viral endocytosis. As presently understood, adenovirus enters cells, e.g., in the respiratory tract, by attaching via the fiber to a cell surface receptor (known as CAR for Coxsackie adenovirus receptor) on the cell membrane of the host cell. The virus thus attached to its receptor migrates into the cell, within the plasma membrane to clathrin-coated pits, which form endocytic vesicles or receptosomes (Shenk, T., "Adenoviridae and Their Replication", in Virology, 2nd ed., Fields et al., eds., Raven Press, New York, 1996). The carboxy-terminus knob portion of the fiber protein functions as the ligand that binds to its cellular receptor (Xia et al., Curr. Top. Micro. Immunol. 199:40-46, 1995; Xia et al., Structure 2:1259-1270, 1995; Henry et al., J.Virol. 68:5239-5246, 1994; Roelvink et al., J.Virol. 70:7614-7621, 1996; Fender et al., Virology 214:110-117, 1995).

It has been determined that alpha-integrins often recognize short amino acid sequences on other cellular proteins for attachment purposes, including the tripeptide sequence Arg-Gly-Asp (abbreviated RGD). An RGD sequence is also found in the penton base protein of adenovirus and is currently understood in the art to mediate the interaction of adenovirus with alpha-integrins (Mathias et al., J.Virol. 68:6811-6814, 1994; Wickham et al., J.Cell Biol. 127:257-264, 1994; Wickham et al., Cell 73:309-319, 1993; Goldman et al., J. Virol. 69:5951-5958, 1995). Once inside the cell, viral particles are transported to the nuclear membrane, where the viral DNA is released from the virion and enters the nucleus through the nuclear pores. Hexon proteins remain associated with the viral DNA in relatively intact particles up until the time of release of the DNA into the cell nucleus. Delivery of viral particles and viral DNA to a target cell is, therefore, largely dependent on the integrity of the individual capsid proteins.

There have been a number of attempts to modify the surface proteins of adenoviral vectors in order to expand their infectious capacility and target cell range.

Modifications of the capsid proteins of adenovirus include those in PCT International Application No. WO97/20051, published June 5, 1997 and PCT International Application No. WO97/05266, published February 13, 1997.

Specific modifications to the penton protein include those in United States Patent No. 5,559,099, issued September 24, 1996, U.S. Patent No. 5,731,190 issued March 24, 1998, and Wickham et al., Gene Ther. 2:750-756, 1995.

Specific modifications to the hexon protein of adenovirus include those in Crompton et al., J. Gen Virol. 75:133-139, 1994, PCT International Publication No. WO98/40509 published September 17, 1998, and PCT International Publication No. WO98/32842 published July 30, 1998.

Modifications of the adenovirus fiber protein include those in PCT International Application No. WO96/26281 published August 29, 1996; United States Patent No. 5,543,328, issued August 6, 1996; Michael et al., Gene Ther. 2:660-668, 1995; Douglas et al., Nature Med. 14:1574-1578, 1996; Wickham et al., Nature Med. 14:1570-1573, 1996; Gall et al., J.Virol. 70:2116-2123, 1996; Stevenson et al., J.Virol. 71:4782-4790, 1997; and Krasnykh et al., J.Virol. 70:6839-6846, 1996; PCT International Application No. WO98/41618 published September 24, 1998; PCT International Application No. WO98/07877 published February 26, 1998; PCT International Application No. WO98/07865 published February 26, 1998; PCT International Application No. WO97/20575 published June 12, 1997; U.S. Patent No. 5,770,442 issued June 23, 1998; and U.S. Patent No. 5,756,086 issued May 26, 1998.

Although adenoviral vectors are currently in clinical trials and have shown the ability to transfer genes to target cells and tissues for expression of the delivered gene, a need remains to improve the infection efficiency of these vectors in order to further improve their gene transfer capabilities and/or to optimize the infection of specific target cells. It would be desirable to identify specific ligands which can confer infectious capability to adenoviral vectors for specific target cells of interest, and to provide adenoviral vectors which comprise such ligands. The present invention addresses this goal.

Summary of the Invention

The present invention is directed to adenoviral vectors having modified capsid proteins which comprise heterologous ligands that improve and or alter the infectious capability of the vectors. Such ligands are capable of binding to desired target cells, and their inclusion into adenoviral vectors facilitates the binding and infectious properties of these vectors. In a preferred embodiment, the ligands are peptides and the target cells are epithelial cells. The invention is also directed to compositions comprising the adenoviral vectors of the invention. Additional aspects of the invention include methods for using the adenoviral vectors of the invention to deliver transgenes to target cells.

Brief Description of the Drawings

Figure 1A shows substitutions in adenovirus hexon protein of heterologous ligands. Figure 1B shows infection of 293 cells with adenoviral vectors with modified hexon proteins. Figure 1C shows infection of CHO cells with adenoviral vectors with modified hexon proteins. Figure 1D shows a graph of transgene expression in CHO cells using adenoviral vectors with modified hexon proteins.

Figure 2A shows a schematic diagram of the adenovirus fiber protein. Figure 2B shows the trimerization capacity of modified fiber proteins.

Figure 3A shows a flow diagram of the protocol used in the biopanning of human airway epithelial cells. Figure 3B shows the consensus amino acid sequences of peptide ligands identified from the biopanning of the human airway epithelial cells.

Figure 4 shows graphs displaying the binding of phage displaying specific peptides to specific cell types: A: normal human bronchial epithelial cells; B: small airway epithelial cells; C: HeLa cells; D: COS cells.

Figure 5A shows cellular pseudo-stratification and ciliagenesis of differentiated normal human bronchial epithelial cells. Figure 5B shows the elution profile of phage displaying specific peptides and binding to normal human bronchial epithelial cells on an air-liquid interface. Figure 5C shows the elution profile of phage displaying specific peptides and binding to differentiated monkey airway epithelial cells.

Figure 6A shows a graph of the elution profiles for phage displaying specific peptides and binding to normal human bronchial epithelial cells on an air-liquid interface.

Figure 6B shows the results of the anti-phage antibody staining on normal human bronchial epithelial cells binding phage displaying specific peptides.

Figure 7 shows the transduction of well-differentiated human airway cells by adenoviral vectors with modified hexon proteins.

Figure 8 shows the transduction of mouse lung cells by adenoviral vectors with modified hexon proteins.

Figure 9 shows a plot of assay results from transduction of mice by adenoviral vectors with modified hexon proteins.

Detailed Description of the Invention

The present invention is directed to adenoviral vectors having modified capsid proteins which comprise heterologous ligands that improve and/or alter the infectious capability of such vectors. Such ligands are capable of binding to target cells, and their inclusion into adenoviral vectors facilitates the binding and infectious properties of the vectors. In a preferred embodiment, the ligands are peptides and the target cells are epithelial cells. The invention is also directed to the novel heterologous ligands, to the oligonucleotides encoding such molecules, and to complexes of a capsid protein of the invention and a cellular receptor which binds the heterologous ligand.

The capsid proteins of adenovirus are defined as the fiber, hexon, penton and protein IX proteins. A heterologous ligand in an adenoviral vector of the invention is defined as a peptide or the amino acid sequence of such peptide which is not native to the adenovirus genome, or as a peptide or the amino acid sequence of such peptide which is native to the adenoviral genome but which is inserted into a heterologous site in the genome. A heterologous ligand of the invention is also defined as an amino acid sequence which substantially corresponds to the amino acid sequence of an identified ligand, or which is an analog or homolog of such ligand.

As used herein, the term "peptide" refers to an oligomer of at least two contiguous amino acids, linked together by a peptide bond., and not greater than fifty amino acids. As used herein, the term "polypeptide" refers to an oligomer of at least fifty amino acids.

As used herein, "substantially corresponds" means an amino acid sequence of a ligand having approximately 70% identity in amino acid sequence to a heterologous

ligand peptide or polypeptide, whether colinear or including gaps in the parent sequence, and which retain the functional capability of the parent peptide. Methods for characterizing identity relationships among two or more amino acid sequences can include the use of algorithms (e.g., as decribed in Molecular Sequence Comparison and Alignment, in Nucleic Acid and Protein Sequence Analysis, Bishop, M. et al., eds., IRL Press, Oxford 1987).

By "homolog" is meant the corresponding peptides or polypeptides from other organisms, so long as the structural and functional properties of the peptides are retained.

By "analog" is meant substitutions, rearrangements, deletions, truncations and additions in the amino acid sequence of a heterologous ligand, so long as the structural and functional properties of the ligands are retained. Analogs also include ligands which contain additional amino acids added to either end of the peptides that do not affect biological activity, e.g., the presence of inert sequences added to a functional ligand which are added to prevent degradation. In another embodiment, conservative amino acid substitutions can be introduced into a ligand provided that the functional activity of the ligand is retained.

The criticality of particular amino acid residues in a ligand of interest may be tested by altering or replacing the residue of interest. For example, the requirement for a cysteine residue at a particular site in the ligand, which can be involved in the formation of intramolecular or intermolecular disulfide bonds, can be tested by mutagenesis of the cysteine to another amino acid, for example, tyrosine, which cannot form such a bond.

In one embodiment of the invention, peptide ligands which are known to bind to target cells can be inserted into the capsid proteins of the adenoviral vectors of the invention. Such ligands include the following: Ad2/5 RGD (containing the RGD sequence from adenovirus serotypes 2 and 5, HAIRGDTFA) (SEQ ID NO. 1) Ad17 RGD (containing the RGD sequence from adenovirus serotype 17, GPARGDSSV) (SEQ ID NO. 2) and the SV40 nuclear localization signal (SV40NLS) (PKKKRKV) (SEQ ID NO. 3) (Kalderon et al., Cell 39:499-509, 1984). Other known ligands which bind to specific receptors on target cells, including RGD sequences from other adenovirus serotypes, or ligands which are involved in nuclear entry pathways can be used to generate adenoviral vectors which are capable of infecting specific target cells. In one embodiment of the

invention, the RGD sequences normally found in the penton protein are inserted into either the fiber or hexon proteins of adenoviral vectors to enhance their binding capability. In a preferred embodiment of the invention, a capsid protein comprises a ligand having an Ad17 RGD sequence: GPARGDSSV (SEQ ID NO. 2)

In another embodiment of the invention, novel ligands which are capable of binding to desired target cells can be inserted into adenoviral capsid proteins. Such ligands can be identified by, for example, phage biopanning techniques (Smith et al., Science 228:1315-1317, 1985; Parmley et al., Gene 73:305-318, 1988; Scott et al., Science 249:386-390, 1990), in which phage engineered to display specific peptides on their surface are incubated with desired target cells to select those phage which bind to the target cells. The peptide contained on the phage is identified and sequenced, and is characterized as a ligand for the particular cell type. Biopanning can be performed by using, for example, a phage library which displays surface peptides and incubating such phage with the desired target cells to identify those phage displaying peptides which are capable of binding to the cells. Phage libraries can be purchased from, for example, New England Biolabs (Beverly, MA) . Biopanning of target cells can be performed in solution on an air-liquid interface (ALI) . Cells grown on an ALI differentiate in a pseudo-stratified layer, with a histology resembling *in vivo* airway epithelia (Gray et al., 1996, Am. J. Respir. Cell Mol. Biol. 14:104-112; Yamaya et al., Am. J. Physiol. 262:L713-724, 1992).

Other methods to identify such peptides which bind to surface cellular receptors can be used, such as incubation of target cells with labelled peptides to identify cells which bind such peptides, or using peptides incubated with cells containing a high abundance of a known receptor in order to more readily isolate peptides which bind to such a receptor. Other methods, such as *in vitro* assays using a cellular receptor bound to a column, for example, to isolate peptide ligands, which can then be incorporated into adenoviral vectors, are known to those skilled in the art. Cells used in the identification of peptide ligands of interest to be inserted into the capsid proteins of adenovirus can be chosen with reference to the target cell or tissue of interest for infection. For example, where the desired target cells are epithelial cells, normal human bronchial epithelial cells (NHBE) or small airway epithelial cells (SAEC) can be used.

Specific novel peptides of the invention which can be inserted into the capsid proteins of the adenoviral vectors invention include the following:

SSS.10	TTDFYYALRALA	SEQ ID NO. 4
SSS.14	TTDFYYALRALA	SEQ ID NO. 5
SSS.8	LPKMASVQRNLA	SEQ ID NO. 6
SSS.9	HETFYSMIRSLA	SEQ ID NO. 7
SSS.5	HDTFLYGLQRLV	SEQ ID NO. 8
SSS.6	LTFDQTPLTAQI	SEQ ID NO. 9
SSS.7	ITFNQTVTTSYM	SEQ ID NO. 10
SSS.16	ETFSDPLAGSSS	SEQ ID NO. 11
SSS.17	SDQLASPYSHPR	SEQ ID NO. 12
polyK	KGKGKGKGKGKG	SEQ ID NO. 13

Preferred novel peptide ligands of the invention for insertion into adenoviral capsid proteins are sss.10 and sss.17.

Other peptides which can be inserted into adenoviral capsid proteins are within the scope of the invention provided they function to enhance viral binding and/ or infectivity in target cells.

The peptide ligands of the invention can be inserted into the fiber, hexon, penton and protein IX proteins of an adenoviral vector, or a vector may contain any combination of such modifications. Preferred insertion sites for the heterologous ligands of the invention are the adenoviral fiber or hexon protein. In a preferred embodiment, one or more heterologous ligands are inserted into the knob region of a fiber protein of an adenoviral vector. The ligands can be inserted into the capsid proteins without the removal of endogenous amino acid sequences or, alternatively, may be inserted in the place of deleted amino acid sequences. Preferably, the heterologous ligands are substituted for wild-type sequence in the proteins in order to maintain the conformational integrity of the capsid protein. Determination of the length of a peptide ligand for insertion into a capsid protein is made with reference to the size of an identified ligand, the site of insertion, including three-dimensional analysis, and the desired target cell.

Preferred sites in the hexon protein of an adenoviral vector for the insertion of one or more ligands to enhance infectivity are the hypervariable regions (1-7) in the hexon

protein (Crawford-Miksza et al., J. Virol. 70:1836-1844, 1996). In the most preferred embodiments, the ligands are inserted into loop 1 (hypervariable region 5) and/or into loop 2 (hypervariable region 7) (Crawford-Miksza et al., J. Virol. 70:1836-1844, 1996) of the hexon protein. Because each modification in the hexon protein is repeated 720 times, the peptide ligand enhancement is greatly amplified.

Preferred sites in the fiber protein of an adenoviral vector for the insertion of one or more ligands to enhance infectivity are in the knob region of the protein (see Zia et al., Structure 21:1259-1270, 1994), which is the carboxy terminus of the protein. For example, peptide ligands can be inserted into the conserved A-J regions, the blade regions of the fiber protein (such as the G, H, I and D regions), or any regions of the knob which mediate cellular interactions (see Xia et al., Curr. Top. Micro. Immunol. 199: 40-46, 1995; Xia et al., Structure 2: 1259-1270, 1995). A preferred site for the insertion of a peptide ligand of the invention is the G region.

The fiber protein can also be modified by altering the number of repeat nucleotide sequences in the shaft of the protein. Such modifications can be used, for example, in combination with hexon proteins containing the ligands of the invention. Where the hexon protein contains ligands of the invention, reduction of the fiber protein shaft may expose the ligand more readily for interaction with the cell surface and enhance or facilitate the infectivity of an adenoviral vector containing both modifications. Trimerization of the fiber protein, which is essential for infectivity, can occur provided that the first residue of the 22nd repeat of the fiber shaft is present (Henry et al., J.Virol. 68:5239-5246, 1994).

The invention is further directed to the cellular receptors for the heterologus ligands of the invention, and to the complexes formed between the ligands and their receptors. One skilled in the art can readily identify the receptors for the peptides of the invention using conventional techniques such as, for example, incubation of labelled peptides with cellular extracts to identify one or more proteins that bind to the receptor or to use a peptide containing a reactive group (for example, cysteine with a free sulfhydryl group) on a resin to isolate one or more receptors from a cellular extract. Reverse genetic techniques which are known to those skilled in the art can be used to identify the genes encoding the receptors so identified.

The invention is also directed to adenoviral vectors which comprise a heterologous DNA sequence of interest (transgene) operably linked to expression control sequences and further comprise one or more modified capsid proteins of the invention. This DNA sequence of interest can be characterized as a transgene. Specific adenoviral vectors into which the modified capsid proteins of the invention can be engineered are disclosed by Zabner et al., Cell 75 : 207, 1993; Zabner et al., J. Clin. Invest. 6 : 1504, 1996; Armentano et al., J. Virol. 71:2408-2416, 1997; Scaria et al., J. Virol. 72:7302, 1998; Kaplan et al., Human Gene Ther. 9:1469-1479, 1998; and U.S. Patent No. 5,670,488 issued September 23, 1997, U.S. Patent No. 5,707,618 issued January 13, 1998, and U.S. Patent No. 5,824,544, issued October 20, 1998, the disclosures of which are incorporated by reference. Adenoviral vectors of the invention may include deletion of the E1 region, partial or complete deletion of the E4 region, and deletions within, for example, the E2 and E3 regions. Adenoviral vectors which comprise a heterologous DNA/transgene of interest, and associated regulatory elements, flanked by the adenoviral inverted terminal repeats and packaging sequences, (as provided in allowed U.S. Patent Application Serial No. 08/895, 194) are also within the scope of the invention as candidates for the insertion of modified capsid proteins. The adenoviral vectors of the invention are preferably replication-defective, that is, they are incapable of generating a productive infection in the host cell.

In preferred embodiments, adenoviral vectors can also be constructed using adenovirus serotypes from the well-studied group C adenoviruses, especially Ad2 and Ad5. Ad17 is also a preferred serotype. However, the design of the adenoviral vectors of the invention using other group C or non-group C adenoviruses is also within the scope of the invention, including the design of chimeric adenviral vectors which contain nucleotide sequences from one or more serotypes. Within the scope of the invention are also, for example, chimeric vectors which contain a genome of a particular serotype and one or more capsid proteins from other serotypes, such as for example, those disclosed in allowed U.S. Application Serial No. 08/752,760 and in PCT Application PCT/US97/21494, filed November 20, 1997. Adenoviral vectors which are chimeric for the capsid proteins are also within the scope of the invention, such as where the fiber and hexon proteins are from different serotypes. In another embodiment, a particular capsid

protein may itself be a chimera, such as a fiber protein which has a modular composition such that the tail, shaft and knob regions may be derived from one or more serotypes. In one preferred embodiment of the invention, an adenoviral vector comprises a fiber protein which has a tail and knob region from serotype Ad2 and a shaft region from serotype Ad17.

In order to construct the adenoviral vectors of the invention, reference may be made to the substantial body of literature on how such vectors may be designed, constructed and propagated using techniques from molecular biology and microbiology that are well-known to the skilled artisan. For example, the skilled artisan can use the standard techniques of molecular biology to engineer a heterologous DNA/transgene operably linked to appropriate regulatory elements into a backbone vector genome and to engineer a ligand into a capsid protein (Berkner, K.L., Curr. Top. Micro. Immunol. 158:39-66, 1992). For example, a plasmid containing a transgene and any regulatory elements of the invention inserted into an adenovirus genomic fragment can be co-transfected with a linearized viral genome derived from an adenoviral vector of interest into a recipient cell under conditions whereby homologous recombination occurs between the genomic fragment and the virus. Preferably, a transgene and any regulatory elements are engineered into the site of an E1 deletion. As a result, the transgene is inserted into the adenoviral genome at the site in which it was cloned into the plasmid, creating a recombinant adenoviral vector. The adenoviral vectors can also be constructed using standard ligation techniques, for example, by engineering a desired restriction site into a capsid protein, allowing for the insertion of a desired oligonucleotide encoding a peptide ligand of interest. Peptides can also be synthesized by standard techniques of protein or peptide synthesis, and may be composed of linear or cyclic peptides.

Construction of the adenoviral vectors can be based on adenovirus DNA sequence information widely available in the field, e.g., nucleic acid sequence databases such as GenBank.

Preparation of replication-defective adenoviral vector stocks can be accomplished using cell lines that complement viral genes deleted from the vector, e.g., 293 or A549 cells containing the deleted adenovirus E1 genomic sequences. HER3 cells (human embryonic retinoblasts transformed by Ad 12), or vK2-20 cells can also be used. After

amplification of plaques in suitable complementing cell lines, the viruses can be recovered by freeze-thawing and subsequently purified using cesium chloride centrifugation. Alternatively, virus purification can be performed using chromatographic techniques, e.g., as set forth in International Application No. PCT/US96/13872, filed August 30, 1996, incorporated herein by reference.

Titers of replication-defective adenoviral vector stocks can be determined by plaque formation in a complementing cell line, e.g., 293 cells. End-point dilution using an antibody to the adenoviral hexon protein may be used to quantitate virus production or infection efficiency of target cells (Armentano et al., Hum. Gene Ther. 6:1343-1353, 1995, incorporated herein by reference).

In another embodiment of the invention, the adenoviral vectors containing modified capsid proteins further comprise nucleotide sequences coding for one or more transgenes. A transgene is identified as a gene which is exogenously provided to a cell by any method of gene transfer. Transgenes which can be delivered and expressed from an adenoviral vector of the invention include, but are not limited to, those encoding enzymes, blood derivatives, hormones, lymphokines such as the interleukins and interferons, coagulants, growth factors, neurotransmitters, tumor suppressors, apoliproteins, antigens, and antibodies, and other biologically active proteins. Specific transgenes which may be encoded by the adenoviral vectors of the invention include, but are not limited to, cystic fibrosis transmembrane regulator (CFTR), dystrophin, glucocerebrosidase, tumor necrosis factor, p53, p21, herpes simplex thymidine kinase and gancyclovir, retinoblastoma (Rb), and adenosine deaminase (ADA). Transgenes encoding antisense molecules or ribozymes are also within the scope of the invention. The vectors may contain one or more transgenes under the control of one or more regulatory elements.

In addition to containing the DNA sequences encoding one or more transgenes, the adenoviral vectors of the invention may contain any expression control sequences such as a promoter or enhancer, a polyadenylation element, and any other regulatory elements that may be used to modulate or increase expression, all of which are operably linked in order to allow expression of the transgene. Viral or non-viral promoters can be operably linked to a transgene in an adenoviral vector, including the CMV promoter or

functional variants thereof. The use of any expression control sequences, or regulatory elements, which facilitate expression of the transgene is within the scope of the invention. Such sequences or elements may be capable of generating tissue-specific expression or be susceptible to induction by exogenous agents or stimuli. Preferred regulatory elements in the adenoviral vectors of the invention include the K18, K14, human β-actin, BOS (EF-1α), ubiquitin B and mucin promoters, the CMV enhancer/promoter, CMV enhancer/E1A promoter, hybrid intron (HI) (Yew et al., Hum. Gene Ther. 8:575-584, 1997) and α-globin stability element (αSE). In a particularly preferred embodiment of the invention, the K18 promoter and the α-globin stability element are used as regulatory elements for expression of a transgene in an adenoviral vector which comprises one or more modified capsid proteins according to the invention.

Infection of target cells by the adenoviral vectors of the invention may also be facilitated by the use of cationic molecules, such as cationic lipids as disclosed in PCT Publication No. WO96/18372, published June 20, 1996, incorporated herein by reference. Adenoviral vectors complexed with cationic molecules are also described in PCT Publication No.WO98/22144, published May 28, 1998, incorporated herein by reference.

Cationic amphiphiles have a chemical structure which encompasses both polar and non-polar domains so that the molecule can simultaneously facilitate entry across a lipid membrane with its non-polar domain while its cationic polar domain attaches to a biologically useful molecule to be transported across the membrane.

Cationic amphiphiles which may be used to form complexes with the adenoviral vectors of the invention include, but are not limited to, cationic lipids, such as DOTMA (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417, 1987) (N-[1-(2,3-dioletloxy)propyl]-N,N,N-trimethylammonium chloride); DOGS (dioctadecylamidoglycylspermine) (Behr et al., Proc. Natl. Acad. Sci. USA 86:6982-6986, 1989); DMRIE (1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide) (Felgner et al., J. Biol. Chem. 269:2550-2561, 1994; and DC-chol (3B [N-N', N'-dimethylaminoethane) -carbamoyl] cholesterol) (U.S. Patent No. 5, 283,185 to Epand et al.). The use of other cationic amphiphiles recognized in the art or which come to be discovered is within the scope of the invention.

In preferred embodiments of the invention, the cationic amphiphiles useful to complex with and facilitate transfer of the vectors of the invention are those lipids which are described in PCT Publication No. WO96/18372, published June 20, 1996, and U.S. Patent No. 5,650,096, both incorporated herein by reference. Preferred cationic amphiphiles described herein to be used in the delivery of the plasmids and/or viruses are GL-53, GL-67, GL-75, GL-87 and GL-89, including protonated, partially protonated, and deprotonated forms thereof. Further embodiments include the use of non-T-shaped amphiphiles as described on pp. 22-23 of the aforementioned PCT application, including protonated, partially protonated and deprotonated forms thereof. Most preferably, the cationic amphiphile which can be used to deliver the vectors of the invention is N^4-spermine cholesteryl carbamate (GL-67).

In the formulation of compositions comprising the adenoviral vectors of the invention, one or more cationic amphiphiles may be formulated with neutral co-lipids such as dileoylphosphatidylethanolamine (DOPE) to facilitate delivery of the vectors into a cell. Other co-lipids which may be used in these complexes include, but are not limited to, diphytanoylphosphatidylethanolamine, lyso-phosphatidylethanolamines, other phosphatidylethanolamines, phosphatidylcholines, lyso-phosphatidylcholines and cholesterol. A preferred molar ratio of cationic amphiphile to colipid is 1:1. However, it is within the scope of the invention to vary this ratio, including also over a considerable range. In a preferred embodiment of the invention, the cationic amphiphile GL-67 and the neutral co-lipid DOPE are combined in a 1:2 molar ratio, respectively, before complexing with an adenoviral vector for delivery to a cell.

In the formulation of complexes containing a cationic amphiphile with an adenoviral vector, a preferred range of 10^7 - 10^{10} infectious units of virus may be combined with a range of 10^4 - 10^6 cationic amphiphile molecules/viral particle.

Assays which determine the binding properties of a modified capsid protein or a ligand of the invention can be performed using *in vitro* assays in which the protein or peptide of interest is incubated with target cells of interest and binding to the cells is measured. Biochemical properties relative to infectivity can be assayed, for example, modified fiber proteins can be assayed for their ability to trimerize, essential for infection,

using standard assays for detection of a high molecular weight protein homotrimer by nondenaturing gel electrophoresis.

The infection efficiency of the adenoviral vectors of the invention containing one or more modified capsid proteins may be assayed by standard techniques to determine the infection of target cells. Such methods include, but are not limited to, plaque formation, end-point dilution using, for example, an antibody to the adenoviral hexon protein, and cell binding assays using radiolabelled virus, or expression of a transgene which is delivered by the virus. Improved infection efficiency may be characterized as an increase in infection of at least an order of magnitude with reference to a control virus.

Where an adenoviral vector of the invention encodes a marker or other transgene, relevant molecular assays to determine expression of the gene include the measurement of transgene mRNA, by, for example, Northern blot, S1 analysis or reverse transcription-polymerase chain reaction (RT-PCR). The presence of a protein encoded by a transgene may be detected by Western blot, immunoprecipitation, immunocytochemistry, or other techniques known to those skilled in the art. Marker-specific assays can also be used, such as X-gal staining of cells infected with an adenoviral vector encoding β-galactosidase or a chemiluminescence assay of β-gal expression using commerical kits, such as Galactolight™, manufactured by Tropix, Bedford, MA..

Specific cell lines which can be used to assess the infection efficiency of the adenoviral vectors of the invention include cells which are normally susceptible to adenoviral infection as well as those that are poorly infected or refractory to wild-type adenoviral infection. For example, Chinese hamster ovary (CHO) cells, which are poorly infected by wild-type adenovirus, can be used to test the adenoviral vectors of the invention containing modified capsid proteins in order to determine whether the heterologous ligand facilitates adenovirus binding to a target cell to provide infection enhancement and efficiency. Other cells can be chosen depending on the particular target cell type sought to be infected with the adenoviral vectors. For example, where epithelial cells are the target cells of interest, normal human bronchial epithelial cells (NHBE) or small airway epithelial cells (SAEC) can be used. Specific enhancement of the infection of a specific cell type can be determined by reference to a non-epithelial cell type, for

example. Other cell lines suitable for assaying the vectors of the invention include HeLa, HUVEC, other established cell lines, as well as primary cells.

In order to determine infection efficiency and transgene expression *in vivo* using the vectors and compositions of the invention, animal models may be particularly relevant in order to assess transgene expression against a background of potential host immune response. Such a model may be chosen with reference to such parameters as ease of delivery, identity of transgene, relevant molecular assays, and assessment of clinical status. Where the transgene encodes a protein whose lack is associated with a particular disease state, an animal model which is representative of the disease state may optimally be used in order to assess a specific phenotypic result and clinical improvement. However, it is also possible that particular adenoviral vectors of the invention display enhanced infection efficiency only in human model systems, e.g., using primary cell cultures, tissue explants, or permanent cell lines. In such circumstances where there is no animal model system available in which to model the infection efficiency of an adenoviral vector with respect to human cells, reference to art-recognized human cell culture models will be most relevant and definitive.

Relevant animals in which the adenoviral vectors may be assayed include, but are not limited to, mice, rats, monkeys, and rabbits. Suitable mouse strains in which the vectors may be tested include, but are not limited to, C3H, C57Bl/6 (wild-type and nude) and Balb/c (available from Taconic Farms, Germantown, New York).

Where it is desirable to assess the host immune response to vector administration, testing in immunocompetent and immunodeficient animals may be compared in order to define specific adverse responses generated by the immune system. The use of immunodeficient animals, e.g., nude mice, may be used to characterize vector performance and persistence of transgene expression, independent of an acquired host response.

In a particular embodiment where the transgene encodes human cystic fibrosis transmembrane conductance regulator protein (CFTR) which is administered to the respiratory epithelium of test animals, expression of human CFTR may be assayed in the lungs of relevant animal models, for example, C57Bl/6 or Balb/c mice, cotton rats, or Rhesus monkeys. Molecular markers, which may used to determine expression, include

the measurement of CFTR mRNA, by, for example, Northern blot, S1 analysis or RT-PCR. The presence of the CFTR protein may be detected by Western blot, immunoprecipitation, immunocytochemistry, or other techniques known to those skilled in the art. Such assays may also be used in tissue culture where cells deficient in a functional CFTR protein and into which the adenoviral vectors have been introduced may be assessed to determine the presence of functional chloride ion channels - indicative of the presence of a functional CFTR molecule.

The adenoviral vectors of the invention have a number of *in vivo* and *in vitro* utilities. The vectors can be used to transfer a normal copy of a transgene encoding a biologically active protein to target cells in order to remedy a deficient or dysfunctional protein, or to provide a protein not normally found in the cell but of interest with respect to a specific phenotype. The vectors can be used to transfer marked transgenes (e.g., containing nucleotide alterations) which allow for distinguishing expression levels of a transduced gene from the levels of an endogenous gene. The adenoviral vectors can also be used to define the mechanism of specific viral protein-cellular protein interactions that are mediated by specific virus surface protein sequences. The vectors can also be used to optimize infection efficiency of specific target cells by adenoviral vectors by engineering specific peptide ligands relevant to target cells of interest into one or more of the capsid proteins. Where it is desirable to use an adenoviral vector for gene transfer to cancer cells in an individual, an adenoviral vector can be chosen which selectively infects the specific type of target cancer cell and avoids promiscuous infection. Where primary cells are isolated from a tumor in an individual requiring gene transfer, the cells may be tested against a panel of adenoviral vectors to select a vector with optimal infection efficiency for gene delivery. The vectors can further be used to transfer tumor antigens to dendritic cells which can then be delivered to an individual to elicit an anti-tumor immune response. The adenoviral vectors can also be used to evade undesirable immune responses to particular adenovirus serotypes or recombinant constructs which compromise the gene transfer capability of adenoviral vectors.

The present invention is further directed to compositions containing the adenoviral vectors of the invention which can be administered in an amount effective to deliver one or more desired transgenes to the cells of an individual in need of such

molecules and cause expression of a transgene encoding a biologically active protein to achieve a specific phenotypic result. The cationic amphiphile-virus complexes may be formulated into compositions for administration to an individual in need of the delivery of the transgenes.

The compositions can include physiologically acceptable carriers, including any relevant solvents. As used herein, "physiologically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the compositions is contemplated. The compositions containing the adenoviral vectors having capsid proteins comprising a heterologous ligand of the invention can also be formulated into dry powder complexes for administration.

Routes of administration for the compositions containing the adenoviral vectors having capsid proteins comprising a heterologous ligand of the invention include conventional and physiologically acceptable routes such as direct delivery to a target organ or tissue, intranasal, intravenous, intramuscular, subcutaneous, intradermal, oral and other parenteral routes of administration.

The invention is further directed to methods for using the compositions containing the adenoviral vectors of the invention *in vivo* or *ex vivo* applications in which it is desirable to deliver one or more transgenes into cells such that the transgene produces a biologically active protein for a normal biological or phenotypic effect. *In vivo* applications involve the direct administration of one ore more adenoviral vectors formulated into a composition to the cells of an individual. *Ex vivo* applications involve the transfer of a composition containing the adenoviral vectors directly to autologous cells which are maintained *in vitro*, followed by readministration of the transduced cells to a recipient.

Dosage of the adenoviral vector having capsid proteins comprising a heterologous ligand of the invention to be administered to an individual for expression of a transgene encoding a biologically active protein and to achieve a specific phenotypic result is determined with reference to various parameters, including the condition to be treated, the age, weight and clinical status of the individual, and the particular molecular defect

requiring the provision of a biologically active protein. The dosage is preferably chosen so that administration causes a specific phenotypic result, as measured by molecular assays or clinical markers. For example, determination of the infection efficiency of an adenoviral vector containing the CFTR transgene which is administered to an individual can be performed by molecular assays including the measurement of CFTR mRNA, by, for example, Northern blot, S1 or RT-PCR analysis or the measurement of the CFTR protein as detected by Western blot, immunoprecipitation, immunocytochemistry, or other techniques known to those skilled in the art. Relevant clinical studies which could be used to assess phenotypic results from delivery of the CFTR transgene include PFT assessment of lung function and radiological evaluation of the lung. Demonstration of the delivery of a transgene encoding CFTR can also be demonstrated by detecting the presence of a functional chloride channel in cells of an individual with cystic fibrosis to whom the vector containing the transgene has been administered (Zabner et al., J. Clin. Invest. 97:1504-1511, 1996). Transgene expression in other disease states can be assayed analogously, using the specific clinical parameters most relevant to the condition.

Dosages of an adenoviral vector of the invention which are effective to provide expression of a transgene encoding a biologically active protein and achieve a specific phenotypic result range from approximately 10^8 infectious units (I.U.) to 10^{11} I.U. for humans.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated, each unit containing a predetermined quantity of active ingredient calculated to produce the specific phenotypic effect in association with the required physiologically acceptable carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly depend on the unique characteristics of the adenoviral vector and the limitations inherent in the art of compounding. The principal active ingredient (the modified adenoviral vector) is compounded for convenient and effective administration in effective amounts with the physiologically acceptable carrier in dosage unit form as discussed above.

Maximum benefit and achievement of a specific phenotypic result from administration of the adenoviral vectors of the invention may require repeated administration. Such repeated administration may involve the use of the same adenoviral vector, or, alternatively, may involve the use of different adenoviral vectors which are rotated in order to alter viral antigen expression and decrease host immune response.

The practice of the invention employs, unless otherwise indicated, conventional techniques of protein chemistry, molecular virology, microbiology, recombinant DNA technology, and pharmacology, which are within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Current Protocols in Molecular Biology, Ausubel et al., eds., John Wiley & Sons, Inc., New York, 1995, and Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, PA, 1985.

The invention is further illustrated by the following specific examples which are not intended in any way to limit the scope of the invention.

EXAMPLE 1: Adenoviral vectors with modified hexon proteins with ligand enhancement

Ligands with known interaction for a cellular receptor or a nuclear entry pathway were substituted into the hypervariable domain of hexon loop 1: the RGD sequences from adenovirus types 2 and 5 (Ad2/5 RGD) (SEQ ID NO 1) or type 17 (Ad17 RGD) (SEQ ID NO. 2), as well as the basic stretch of amino acids within SV40 large T antigen that targets that protein to the nucleus (SV40 NLS) (SEQ ID NO. 3) (Figure 1A). Modified hexon proteins were incorporated into adenoviral vectors expressing βgal and used for infections of both established and primary cells.

Hexon proteins were modified by the insertion of oligonucleotides encoding the peptides into newly created restriction enzyme sites within the respective sequences. An AatII site was created in substitution for wild-type nucleotides 19680 through 19740 by changing the flanking nucleotides at the 5' end, TACCTC, to GACGTC and at the 3' end, GATGTA, to GACGTC. Oligonucleotides encoding specific amino acids were then inserted into the AatII site, creating a modified hexon protein with in-frame protein substitutions within loop 1.

293 cells (in DMEM) and CHO cells (in F12-Ham's media) were infected at subconfluency with Ad2-βgal-4 (Armentano et al., J.Virol. 71:2408-2416, 1997) or an identical vector except for the substitution of RGD or SV40NLS peptide sequences within hexon loop 1. 293 cells were infected overnight at a multiplicity of infection (MOI) of 0.5. 24 hours post-infection, the cells were stained for β-gal activity and photographed. CHO cells were infected for 4 hours at an MOI of 50. 48 hours following infection, the cells were stained for β-gal activity and photographed.

Figure 1A): Peptides substituted into the hypervariable domain of hexon loop 1 include the RGD sequences from adenovirus types 2 and 5 (Ad2/5 RGD) or type 17 (Ad17 RGD), as well as the basic stretch of amino acids within SV40 large T antigen that targets that protein to the nucleus (SV40 NLS). The amino acid substitutions within the hexon protein are underlined. Figure 1B): 293 cells were infected overnight with Ad2/bgal-4 (A), Ad2/βgal/hex.mod.Ad2/5RGD (B), Ad2/βgal/hex.mod.Ad17RGD (C), or Ad2βgal/hex.mod.SV40NLS (D) at an MOI = 0.5. Cells were photographed 24 hours post-infection. Figure 1C): CHO cells were infected for 4 hours with Ad2/βgal-4 (A), Ad2/βgal/hex.mod.Ad2/5RGD (B), Ad2/βgal/hex.mod.Ad17RGD (C), or Ad2βgal/hex.mod.SV40NLS (D) at an MOI = 50. Cells were photographed 48 hours post-infection. Cells from similarly infected dishes were quantitated for βgal expression by luminometer. Each bar in the graph represents the average of 3 separate assays on CHO cells from one dish (Figure 1D).

The results indicate that adenoviral vectors modified by the insertion of RGD or SV40NLS peptides in loop 1 of the hexon protein can infect cells with differential efficiency, as a function of the specific peptide ligand inserted into the hexon protein.

EXAMPLE 2: Modification of the adenovirus fiber knob by incorporation of a novel ligand

Oligonucleotides encoding a peptide linker (PGSASGSASGSP) (SEQ ID NO. 20) and a new enzyme site (AatII) were inserted just upstream of the translation termination site of adenovirus DNA encoding the fiber protein (Figure 2A). Oligonucleotides encoding specific amino acids were then inserted into the AatII site, creating a fiber knob with in-frame protein additions just upstream of the translation termination site. The

oligonucleotide additions are flanked by AatII sites, thus adding two amino acids (DV) upstream and downstream of the inserted peptides. The carboxy-terminus of fiber knob was modified by the addition of a linker (-PGSASGSASGSP-) and ligand (sss.10 or sss.17).

Modified fiber knob proteins, from the first residue of the 22nd repeat of the shaft through the stop site, were cloned into the NdeI-BamHI site of pET-15b (Novagen). Each protein was expressed from the T7 promoter in a rabbit reticulocyte lysate (Promega). 1 µl of each translation reaction was added to a standard protein loading buffer with (+) or without (-) DTT as a denaturing reagant and with (+) or without (-) heating. Protein trimerization capacity was verified by the migration of the protein at a high molecular weight (2-3 times the size of the monomer), under nondenaturing conditions, on a 12% polyacrylamide gel.

The results show that novel ligands, having high affinity for human epithelial cells, can be incorporated into the fiber carboxy terminus, while allowing the capacity of the knob to trimerize to be retained (Figure 2B). Lanes 1 and 2, pET-15b vector; lanes 3 and 4, fiber knob/sss.17; lanes 5 and 6, fiber knob/sss. 10; lanes 7 and 8, wild-type fiber knob; M, marker.

EXAMPLE 3: Identification of novel binding peptides

Primary human airway epithelial (NHBE) cells were purchased from Clonetics (San Diego, CA). Frozen cells were spun down gently (1000 rpm for 5' at room temperature) in media or HBSS, then resuspended in blocking buffer for biopanning.

Differentiated, ciliated airway epithelial cells on an air-liquid interface (ALI) were created according to the protocol of Gray et al (Am. J. Respir. Cell Mol. Biol. 14:104-112, 1996). Cell growth medium was supplemented as recommended by the supplier, with the following modifications: 25ng/ml hEGF, $5x10^{-8}$ M retinoic acid and 0.5 mg/ml BSA were added to the media of cells growing on plastic, while 0.5 ng/ml hEGF, $5x10^{-8}$ M retinoic acid, and 1.5 mg/ml BSA were added to the media of cells growing in transwells. Cells on plastic were grown in BEGM medium; cells in transwells were grown in a 1:1 mixture of DMEM (low glucose):BEBM.

Rat tail collagen type 1 (Collaborative Biomedical Products, Waltham, MA) was diluted to 3 mg/ml with 0.02N acetic acid. 100 μl was added to the upper chamber of transwells (Costar transwell clear) in 24-well plates. The plates were then placed, uncovered, in an ammonium vapor chamber for 3' to cross-link the collagen matrix. Upper and lower chambers of the wells were rinsed with 3 changes of sterile water over 2 - 3 hours. Water was replaced by unsupplemented DMEM for 24 hours. Prior to cell seeding, inserts were equilibrated for 2 hours at room temperature with DMEM:BEGM/10%FBS.

Primary NHBE cells were seeded on the collagen gels at 0.5 - 1.0 x 10^5 cells/cm^2 in DMEM:BEGM. Cells were grown submerged until confluency, with media changes 24 hours following seeding and every 48 hours subsequently. For the first 24 hours following seeding, DMEM:BEGM/10%FBS was left in the bottom chamber. Subsequently, DMEM:BEGM was added to both upper and lower chambers. The ALI was created by removing the media from the upper chamber upon cell confluency. Media in the lower chamber (basal surface of cells) was changed daily thereafter.

For cells on ALIs, blocking buffer was added to both the top and bottom chambers for 30'. Block buffer in the top chamber was then replaced with phage in 100 μl block buffer. Following phage binding, the inserts were transferred to separate 50 ml polypropylene tubes, where they were washed 3 successive times with 10 ml block buffer. Phage were eluted by adding 200 μl elution buffer directly to the inner well chambers.

- Phage biopanning. A phage library, displaying linear dodecapeptides fused to protein III, was purchased from New England Biolabs. For biopanning cells in solution, 10^{11} to 10^{13} phage and 10^6 cells were blocked separately in 200 μl blocking buffer (3% BSA, 0.1% hydrolysate casein, 0.02% azide in HBSS without Mg^{++} or Ca^{++}) for 30' with gentle rocking at room temperature. The cells and phage were then combined (400 μl total) and incubated for 1 hour at room temperature, with gentle rocking. The cells bound with phage were spun down at 2000 rpm for 3' in a microcentrifuge, then washed with 3 successive 10 ml rinses of block buffer. Phage were eluted from the cell pellet with 200 μl of 0.12M glycine, pH2.0/0.5% BSA for 5' at room temperature. Debris was removed

by a quick centrifugation. The elution solution was neutralized by the addition of 2 mls 50mM Tris, pH 8.0.

After 3 rounds of biopanning in solution or on ALIs (Figure 3A), eluted phage were used to infect bacterial cells (Supplier's protocol, New England Biolabs) for single plague isolations, DNA extractions, and sequencing. 20 plaques from each infection were sequenced in the region corresponding to the 5' end of gene III, the site of the insertions in the library. The results indicate that 3 rounds of biopanning of primary NHBE cells in solution led to the isolation of phage with similar amino acid sequences (see underlined amino acids). Figure 3B shows the consensus amino acid sequneces from peptide ligands identified from phage which bound the epithelial cells. Sequence identity among various peptides are shown as underlined amino acids. Only the phage from the solution biopanning showed the consensus sequences as shown here.

EXAMPLE 4: Affinity profiles of phage for different cell types

NHBE and SAEC cells for solution binding to phage were used directly from frozen cultures. HeLa and COS cells were grown on plastic (DMEM medium), trypsinized, spun down in media, and resuspended in block buffer for binding to phage. Phage binding was as described in Figure 3, with the exception that the phage used for these studies (sss.10, sss.6, Ad2/5RGD, and S3-21) are monoclonal phage.

Primary human bronchial epithelial NHBE (Figure 4A), primary small airway epithelial cells SAEC (Figure 4B), transformed cervical carcinoma cells HeLa (Figure 4C) or transformed African green monkey kidney fibroblast cells COS (Figure 4D) cells in solution were bound with phage displaying sss.10 or sss.6 peptides, an Ad2/5 RGD ligand, a Fab fragment with high affinity for many cell types (S3-21), or a wild-type phage filament. Bar graphs represent the affinity of each monoclonal phage, as measured by acid elution and subsequent bacterial infection by the phage.

The results show that phage displaying peptides with high affinity for NHBE cells show a similar binding profile for SAEC. Cells of a different tissue from the same or different species (HeLa, and COS, respectively) bind these phage with different relative efficiencies.

EXAMPLE 5: Affinity of phage for differentiated ciliated NHBE cells and monkey airway cells

Monkey airway epithelial cells were isolated by a standard primary cell isolation procedure. In brief summary, airways were dissected and rinsed with DMEM. Lumen of airways were flushed with DMEM, flushed with 0.1% protease, and submerged in protease overnight. Airways were then flushed with MEM/10%FBS. The recovered washes were spun down at 4°C for 15' at 1000 rpm. The cell pellet was resuspended in MEM/10%FBS, and the cells were seeded onto plastic in a 24-well dish.

The air-liquid interfaces were as described in Figure 3. Phage binding and elution was also performed as described in Figure 3, with the exception that the phage are monoclonal.

Primary NHBE cells were seeded on collagen gels in plastic inserts of a 24-well dish, grown to confluence while submerged in media, then transferred to an air/liquid interface. Cellular pseudo-stratification and ciliagenesis occured at 14 - 21 days post-seeding (Figure 5A). Separate inserts were bound with phage displaying high affinity peptides (sss.10, sss.8, sss.16) or wild-type filaments. Each bar in the graphs represents the elution profile of phage bound to a separate collagen insert (Figure 5B).

Differentiated, ciliated monkey airway epithelial cells (from proteolytically digested airways) were seeded in plastic wells, then bound with phage displaying peptides (sss.10, sss.8) or a Fab fragment (N3-14) with high affinity for human airway epithelial cells, or with wild-type phage. Wells without cells ("none") were also bound with phage as a control for non-specific binding to plastic (Figure 5C).

The results show that phage displaying peptides with high affinity for NHBE cells in solution also show high affinities for both human and monkey differentiated, ciliated airway epithelial cells (Figures 5B and 5C).

EXAMPLE 6: Affinity of phage for NHBE cells on ALI

Differentiated, ciliated NHBE cells were bound with monoclonal phage using the biopanning protocol described above. Following washes, inserts were fixed at 4°C for 30' in 2% paraformaldehyde, 0.2% gluteraldehyde in PBS. Following 3-5 rinses in cold PBS, cells were blocked with 2% BSA, 1% fish gelatin, 10% horse serum in PBS for 1

hour at room temperature. Primary antibody (anti-M13, Pharmacia) was added overnight at 4°C. Following 3-5 washes with cold PBS, secondary FITC-labelled antibody was added for 1 hour at 37°C. After 3-5 washes with cold PBS, cells were again fixed. The inserts were mounted on slides for photography. 2 µg/ml DAPI was added for visualization of the nuclei.

Figure 6A shows a graph of the elution profiles for each phage. These results show that sss.10 and sss.17 (the two phage with the highest peptide affinities), and N3-14 (a phage displaying a Fab fragment with high affinity for human epithelial cells) were bound to ALI inserts. The inserts were subsequently washed, fixed, and bound with an anti-phage primary antibody followed by a FITC-labelled secondary antibody. Figure 6B shows the results of the antibody staining on inserts binding phage displaying specific peptides: N3-14, sss.10, sss.17 and wild type control. These results illustrate that phage displaying peptides isolated by biopanning on NHBE cells bind directly and with specificity to differentiated airway epithelial cells.

EXAMPLE 7: Transduction of well-differentiated human airway epithelial cells on air-liquid interfaces by adenoviruses containing modified capsid proteins

Airway epithelial cells were obtained from normal lung donors. Cells were isolated by enzyme digestion as previously described (Zabner et al., J.Clin.Invest. 100:1144-1149, 1997). Freshly isolated cells were seeded at a density of 5×10^5 cells/cm^2 onto collagen-coated permeable membranes (0.6 cm^2/Millipore-Inserts). The cells were maintained at 37°C in a humidified atmosphere of 7% CO_2 and air. Twenty-four hours after plating, the mucosal media was removed and the cells were allowed to grow at the air-liquid interface. The culture medium was a mixture of 49% DMEM, 49% Ham's F12 and 2% Ultraser G (Sepracor Inc., Marlborough, PA). Penicillin 100 U/ml and streptomycin 100 ug/ml were added to the media.

The airway epithelia were then cultured for 14 days at the air-liquid interface. The cells were exposed to 50 MOI of the modified viruses in 50 ul of PBS for 30 min, and then rinsed twice with PBS. Seventy-two hours later, β-galactosidase expression was measured using a commercially available galactocyte assay using AMPGD (3-(4-methoxyspiro [1,2-dioxethane-3,2'-tricyclo-[3.3.1.1^{3,7}] decan]-4-yl) phenyl-β-D-

galactopyranoside) (Galactolight™) assay (Tropix, Bedford, MA). Briefly, after rinsing with PBS, cells were removed from filters by incubation with 120 ul lysis buffer (25 mM Tris-phosphate, pH 7.8, 2 mM DTT, 2 mM 1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid; 10% glycerol; and 1% Triton X-100) for 15 min. Light emission was quantified in a luminometer (Analytical Luminescence Laboratory, San Diego, CA) and expressed as light units (LU).

The results are shown in Figure 7. Substitution of the sss.17 peptide in hexon loop 1 increased transduction of well-differentiated human airway cells 2-fold, in comparison to wild-type vector (Ad2/β-gal-4). Substitution with the Ad17 RGD peptide in the hexon protein increased transduction efficiency 2.5-fold.

EXAMPLE 8: Transduction Efficiency of Mouse Airways with Hexon-Modified Adenoviral Vectors

Balb/c mice were instilled with 5 x 10^8 infectious units (IU) of each adenoviral vector. At 3 days post-instillation, mice were sacrificed to determine β-gal expression in the lungs by X-gal staining and AMPGD analysis, as described in Example 7. The results are expressed in relative light units per microgram protein (RLU/μg).

Substitution of the sss.17 peptide in the hexon loop 1 of an adenoviral vecvtor increases transduction efficiency 2.4-fold, in comparison to wild-type vector (Ad2/β-gal 4), as shown in Figure 8. Substitution with the Ad17RGD peptide in hexon loop 1 increases transduction efficiency 2.5-fold. The results from assays on individual animals and calculation of the mean result from all assays in shown in Figure 9.

Claims

1. An adenoviral capsid protein comprising a heterologous ligand, wherein the ligand facilitates binding of adenovirus to a target cell.

2. The adenoviral capsid protein of Claim 1 which is fiber protein.

3. The adenoviral capsid protein of Claim 1 which is hexon protein.

4. The adenoviral capsid protein of Claim 1 which is protein IX.

5. A heterologous ligand having the amino acid sequence of SEQ ID NO. 2.

6. A heterologous ligand having the amino acid sequence of SEQ ID NO. 12.

7. An oligonucleotide encoding a heterologous ligand having the amino acid sequence of SEQ ID NO. 2.

8. An oligonucleotide encoding a heterologous ligand having the amino sequence of SEQ ID NO. 12.

9. The adenoviral capsid protein of Claim 1 which comprises a heterologous ligand having the amino acid of SEQ ID NO. 2.

10. The adenoviral capsid protein of Claim 1 which comprises a heterologous ligand having the amino acid of SEQ ID NO. 12.

11. The adenoviral capsid protein of Claim 2 which comprises a heterologous ligand having the amino acid of SEQ ID NO. 2.

12. The adenoviral capsid protein of Claim 2 which comprises a heterologous ligand having the amino acid of SEQ ID NO. 12.

13. The adenoviral capsid protein of Claim 3 which comprises a heterologous ligand having the amino acid of SEQ ID NO. 2.

14. The adenoviral capsid protein of Claim 3 which comprises a heterologous ligand having the amino acid of SEQ ID NO. 12.

15. The adenoviral capsid protein of Claim 4 which comprises a heterologous ligand having the amino acid of SEQ ID NO. 2.

16. The adenoviral capsid protein of Claim 4 which comprises a heterologous ligand having the amino acid of SEQ ID NO. 12.

17. An adenoviral vector which comprises a transgene operably linked to

expression control sequences and further comprises one or more capsid proteins of Claim 1.

18. An adenoviral vector which comprises a transgene operably linked to expression control sequences and further comprises the capsid protein of Claim 2.

19. An adenoviral vector which comprises a transgene operably linked to expression control sequences and further comprises the capsid protein of Claim 3.

20. An adenoviral vector which comprises a transgene operably linked to expression control sequences and further comprises the capsid protein of Claim 4.

21. An adenoviral vector which comprises a transgene operably linked to expression control sequences and further comprises the capsid protein of Claim 9.

22. An adenoviral vector which comprises a transgene operably linked to expression control sequences and further comprises the capsid protein of Claim 10.

23. An adenoviral vector which comprises a transgene operably linked to expression control sequences and further comprises the capsid protein of Claim 11.

24. An adenoviral vector which comprises a transgene operably linked to expression control sequences and further comprises the capsid protein of Claim 12.

25. An adenoviral vector which comprises a transgene operably linked to expression control sequences and further comprises the capsid protein of Claim 13.

26. An adenoviral vector which comprises a transgene operably linked to expression control sequences and further comprises the capsid protein of Claim 14.

27. An adenoviral vector which comprises a transgene operably linked to expression control sequences and further comprises the capsid protein of Claim 15.

28. An adenoviral vector which comprises a transgene operably linked to expression control sequences and further comprises the capsid protein of Claim 16.

29. A complex comprising the heterologous ligand of Claim 5 and a cellular receptor which binds thereto.

30. A complex comprising the heterologous ligand of Claim 6 and a cellular receptor which binds thereto.

31. A composition comprising the adenoviral vector of Claim 17 and a carrier.

32. A composition comprising the adenoviral vector of Claim 18 and a carrier.

33. A composition comprising the adenoviral vector of Claim 19 and a carrier.

34. A composition comprising the adenoviral vector of Claim 20 and a carrier.

35. A composition comprising the adenoviral vector of Claim 21 and a carrier.

36. A composition comprising the adenoviral vector of Claim 22 and a carrier.

37. A composition comprising the adenoviral vector of Claim 23 and a carrier.

38. A composition comprising the adenoviral vector of Claim 24 and a carrier.

39. A composition comprising the adenoviral vector of Claim 25 and a carrier.

40. A composition comprising the adenoviral vector of Claim 26 and a carrier.

41. A composition comprising the adenoviral vector of Claim 27 and a carrier.

42. A composition comprising the adenoviral vector of Claim 28 and a carrier.

43. A method for providing a transgene to a target cell, comprising administering an adenoviral vector which comprises the transgene operably linked to expression control sequences and further comprises a capsid protein which itself comprises a heterologous ligand that facilitates the binding of the vector to said target cell.

44. The method of Claim 43, in which the adenoviral vector comprises a transgene operably linked to expression control sequences and further comprises a fiber protein which itself comprises a heterologous ligand that facilitates the binding of the vector to the target cell.

45. The method of Claim 43, in which the adenoviral vector comprises a transgene operably linked to expression control sequences and further comprises a hexon protein which itself comprises a heterologous ligand that facilitates the binding of the vector to the target cell.

46. The method of Claim 43, in which the adenoviral vector comprises a transgene operably linked to expression control sequences and further comprises a capsid protein which itself comprises a heterologous ligand having the amino acid sequence of SEQ. ID NO. 2.

47. The method of Claim 43, in which the adenoviral vector comprises a transgene operably linked to expression control sequences and further comprises a capsid protein which itself comprises a heterologous ligand having the amino acid sequence of SEQ. ID NO. 12.

48. The method of Claim 43, in which the adenoviral vector comprises a

transgene operably linked to expression control sequences and further comprises a fiber protein which itself comprises a heterologous ligand having the amino acid sequence of SEQ. ID NO. 2.

49. The method of Claim 43, in which the adenoviral vector comprises a transgene operably linked to expression control sequences and further comprises a fiber protein which itself comprises a heterologous ligand having the amino acid sequence of SEQ. ID NO. 12.

50. The method of Claim 43, in which the adenoviral vector comprises a transgene operably linked to expression control sequences and further comprises a hexon protein which itself comprises a heterologous ligand having the amino acid sequence of SEQ. ID NO. 2.

51. The method of Claim 43, in which the adenoviral vector comprises a transgene operably linked to expression control sequences and further comprises a hexon protein which itself comprises a heterologous ligand having the amino acid sequence of SEQ. ID NO. 12.

wt L1	...SNTTSLNDRQGNATKPKVVLYSEDVNM..	SEQ ID NO.14
SSS.17	SNTTSSDQLASPYSHPRVVLYSEDVN	SEQ ID NO.15
Ad 17 RGD	...SNTTSGPARGDSSVKPKVVLYSEDVNM..	SEQ ID NO.16
Ad 2/5 RGD	...SNTTSHAIRGDTFAKPKVVLYSEDVNM..	SEQ ID NO.17
SV40 NLS	...SNTTSLPKKKRKVNAPKVVLYSEDVNM..	SEQ ID NO.18
polyK	SNTTSKGKGKGKGKGKGVVLYSEDVN	SEQ ID NO.19

FIG. 1A

FIG. 1B

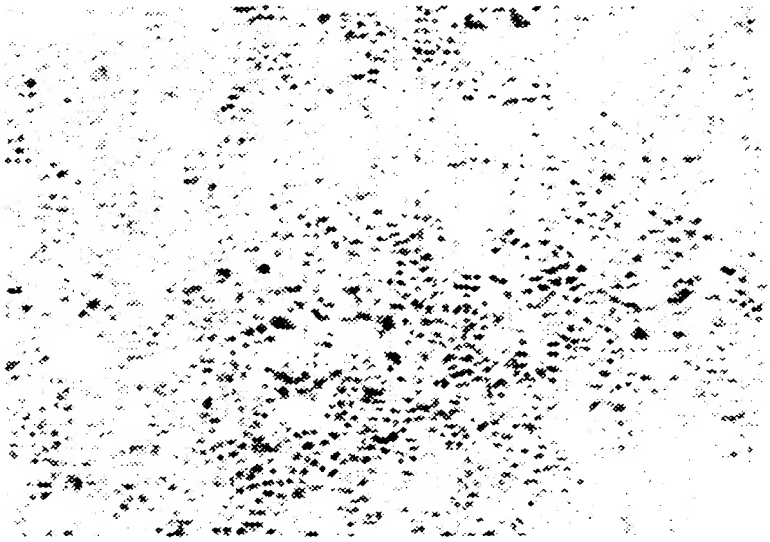


FIG. 1C

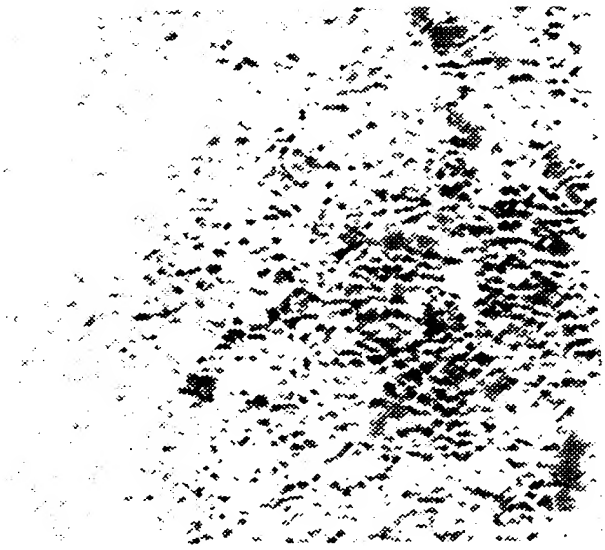


FIG.1D

FIG.1E

FIG.1F

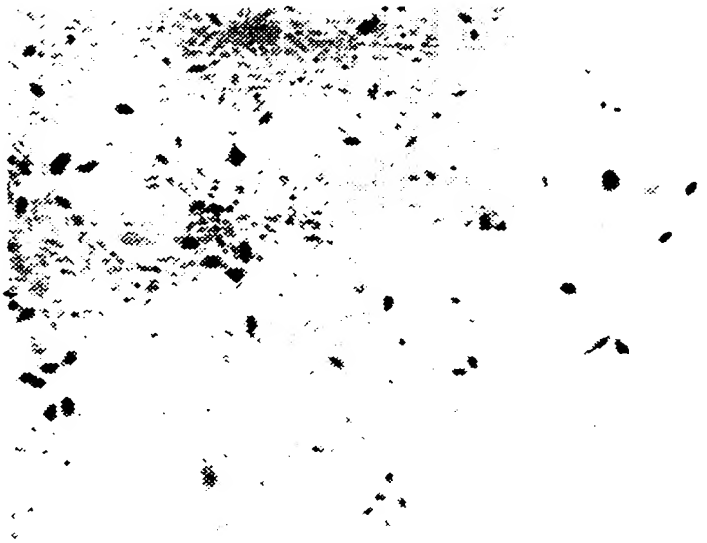


FIG.1G

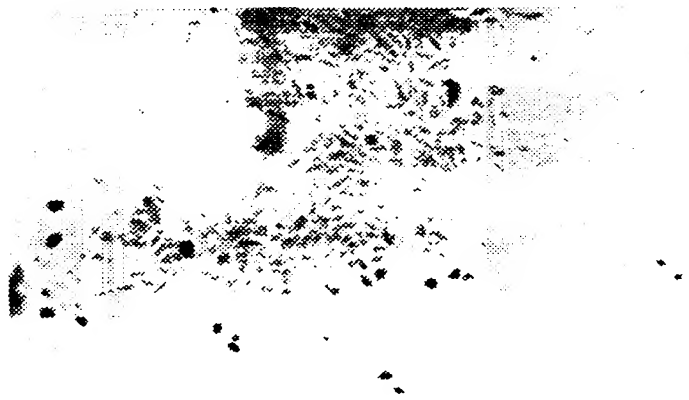


FIG. 1H

FIG. 1I

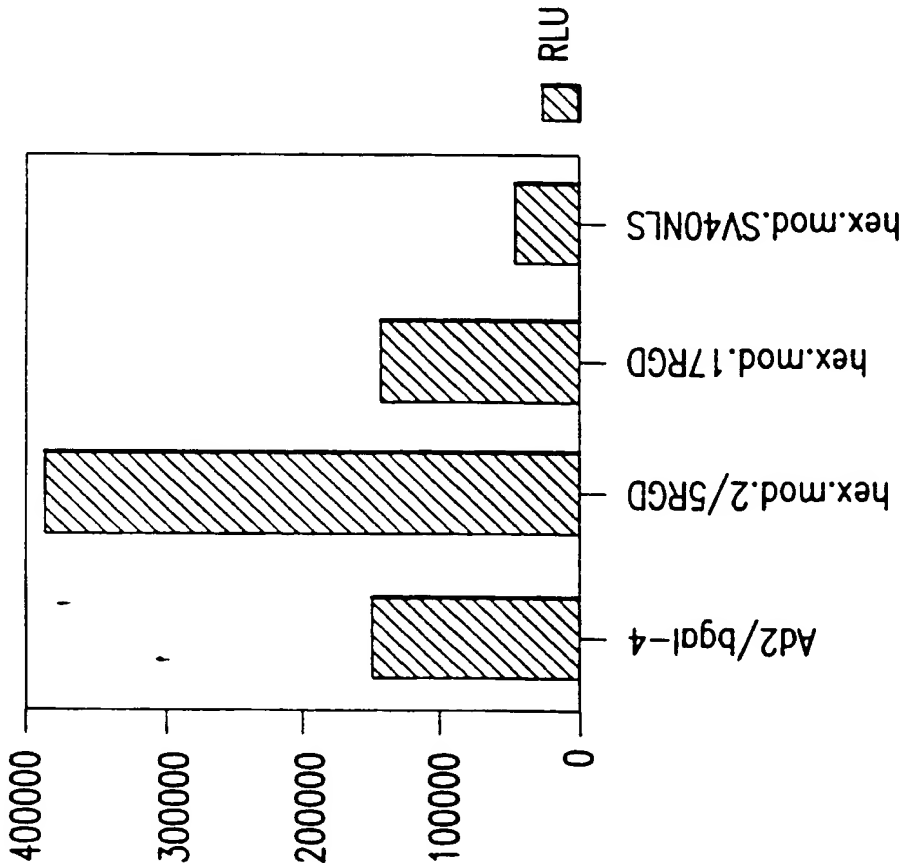

RLU
400000
300000
200000
100000
0
Ad2/bgal-4
hex.mod.2/5RGD
hex.mod.17RGD
hex.mod.SV40NLS
VIRAL VECTOR

FIG.1J

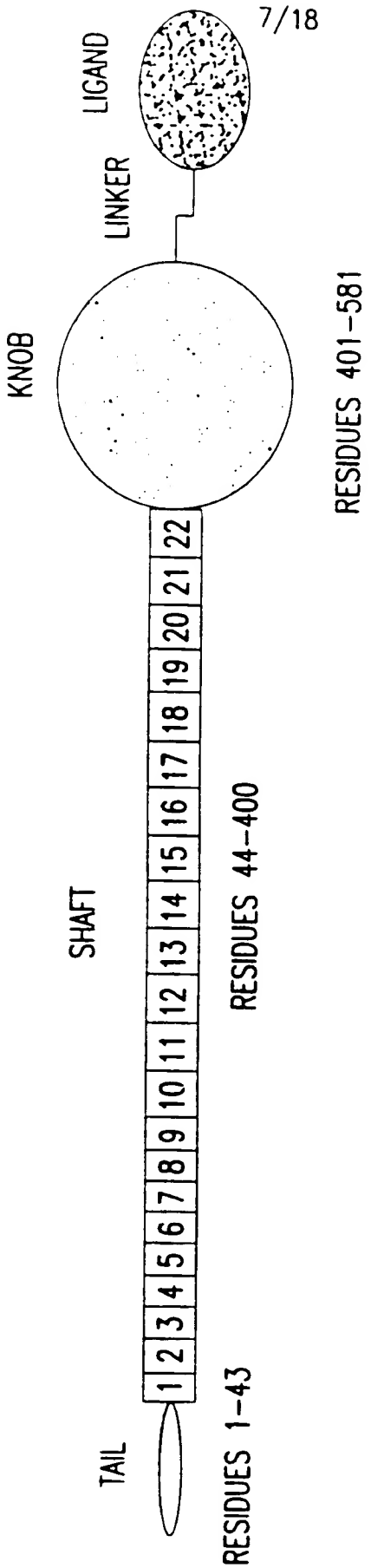

LIGAND
LINKER
KNOB
RESIDUES 401-581
SHAFT
RESIDUES 44-400
TAIL
RESIDUES 1-43
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

FIG.2A

FIG.2B

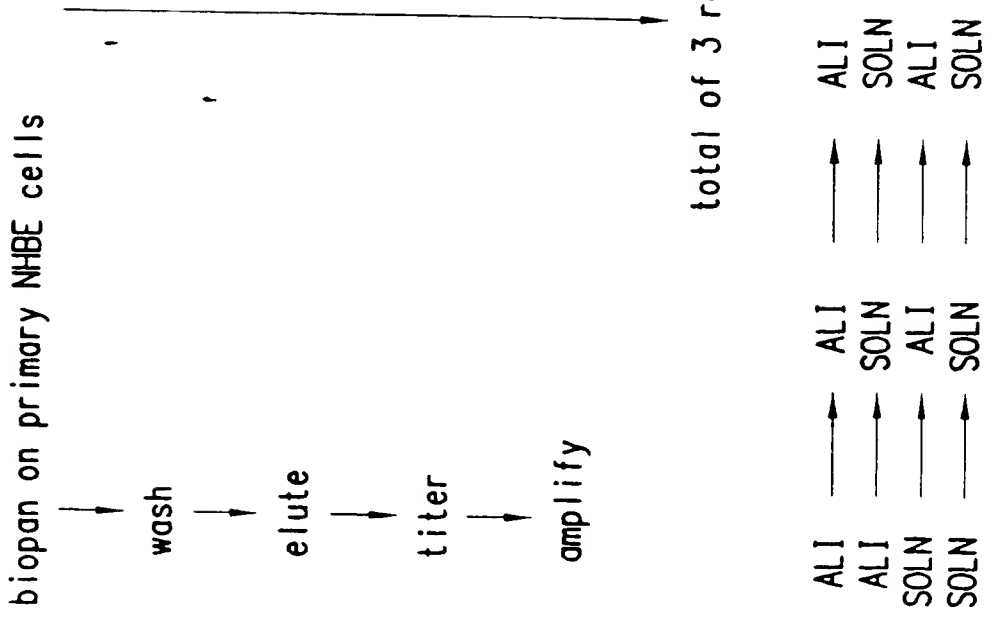


FIG. 3A

consensus sss sequences	
sss.10	TTDFYYALRALA
sss.14	TTDFYYALRALA
sss.8	LPKMASVQRNLA
sss.9	HETFYSMIRSLA
sss.5	HDTFLYGLQRLV
sss.9	HETFTSMIRSLA
sss.6	LTFDQTPLTAQI
sss.7/mt6	ITFNQTVTTSYM
sss.16	ETFSDPLAGSSS
sss.17	SDQLASPYSHPR

FIG. 3B

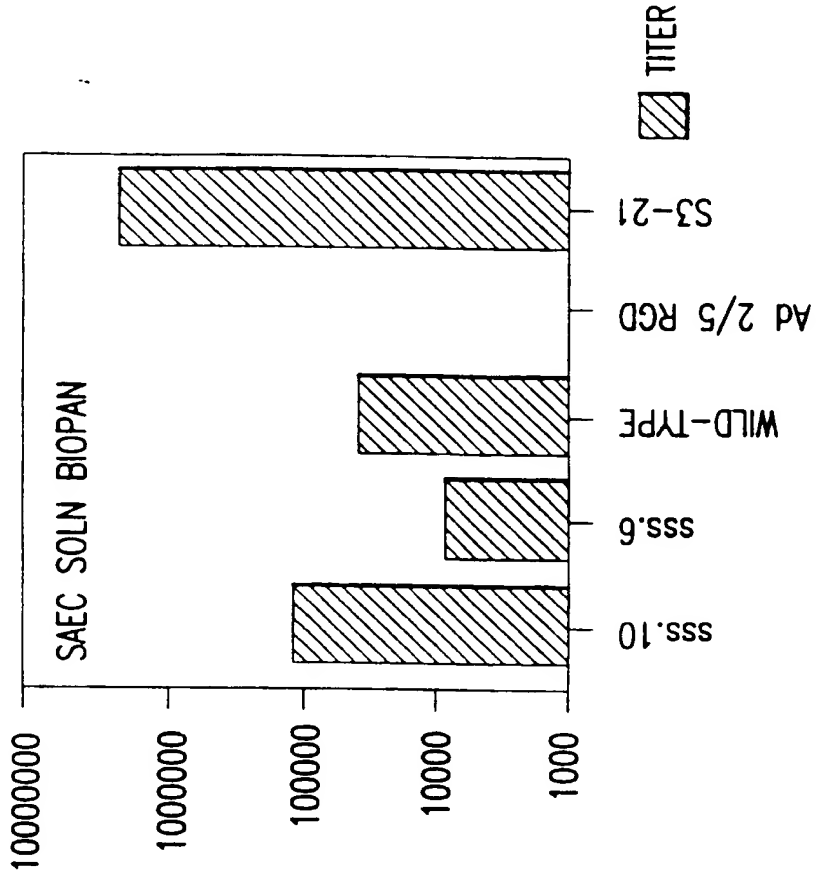

TITER
SAEC SOLN BIOPAN
10000000
1000000
100000
10000
1000
sss.10
sss.6
WILD-TYPE
Ad 2/5 RGD
S3-21

FIG.4B

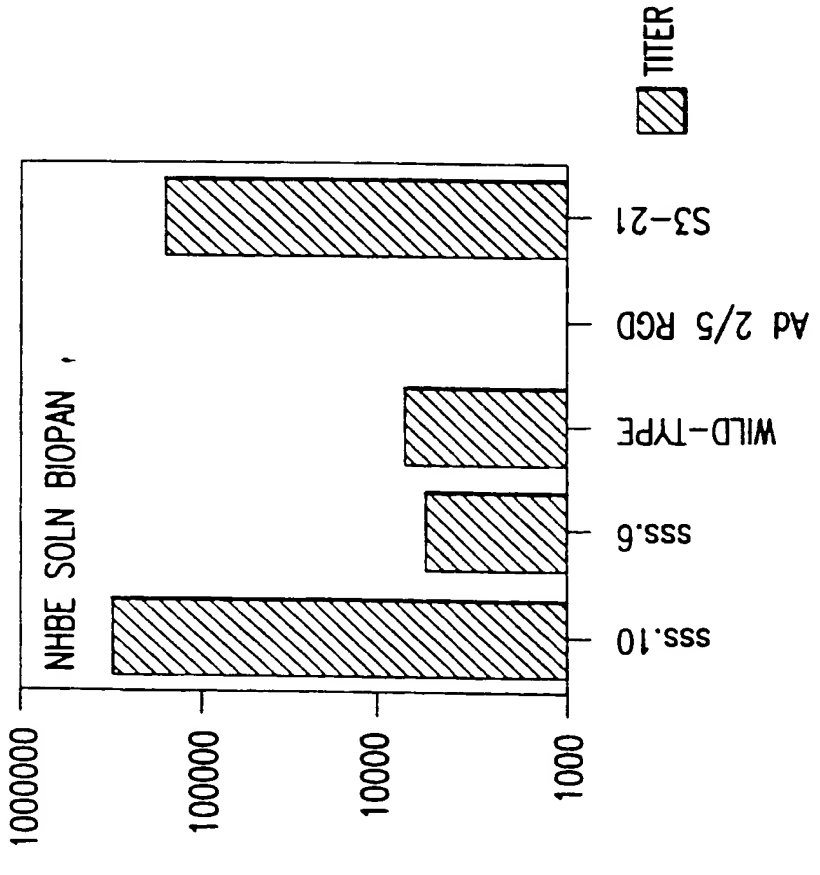
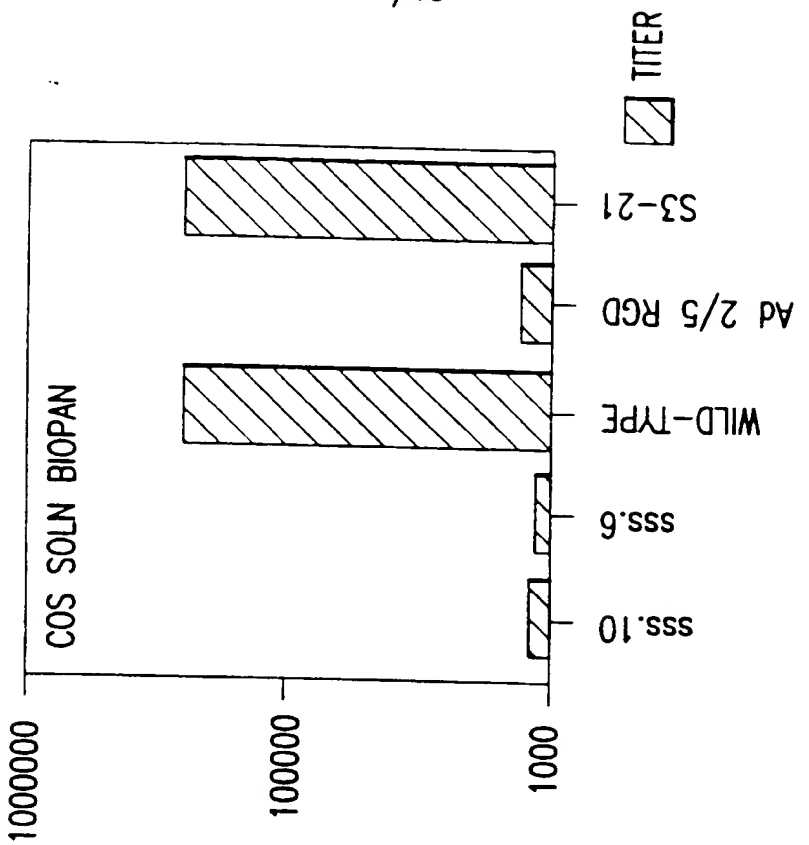

TITER
NHBE SOLN BIOPAN
1000000
100000
10000
1000
sss.10
sss.6
WILD-TYPE
Ad 2/5 RGD
S3-21

FIG.4A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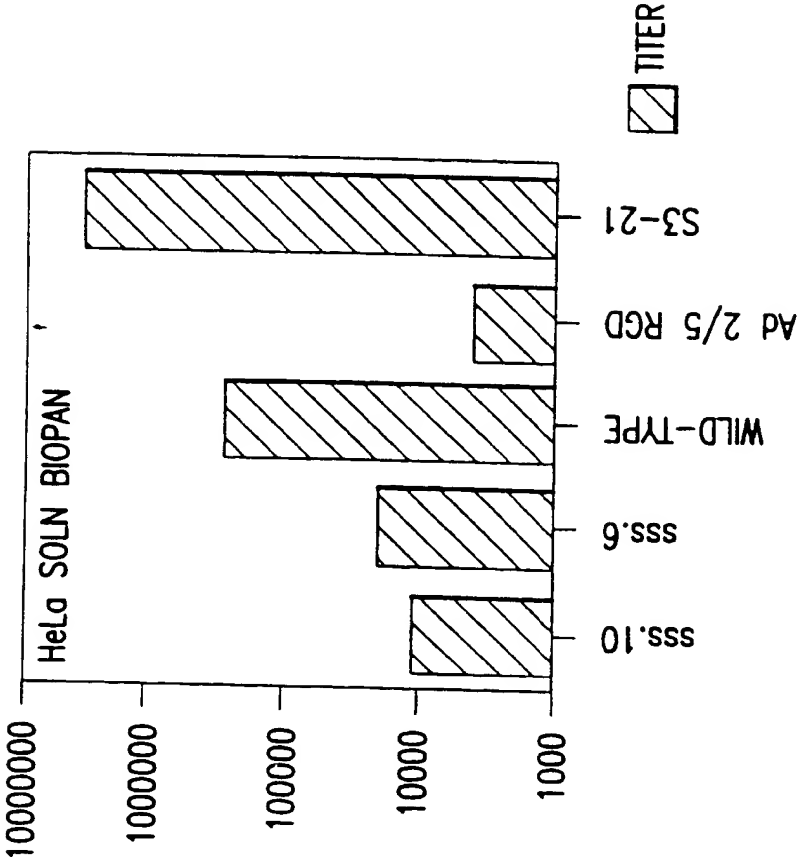
S3-21
Ad 2/5 RGD
WILD-TYPE
sss.6
sss.10
COS SOLN BIOPAN
1000000
100000
1000

FIG.4D


TITER
S3-21
Ad 2/5 RGD
WILD-TYPE
sss.6
sss.10
HeLa SOLN BIOPAN
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1000000
100000
10000
1000

FIG.4C

FIG.5B

FIG.5A

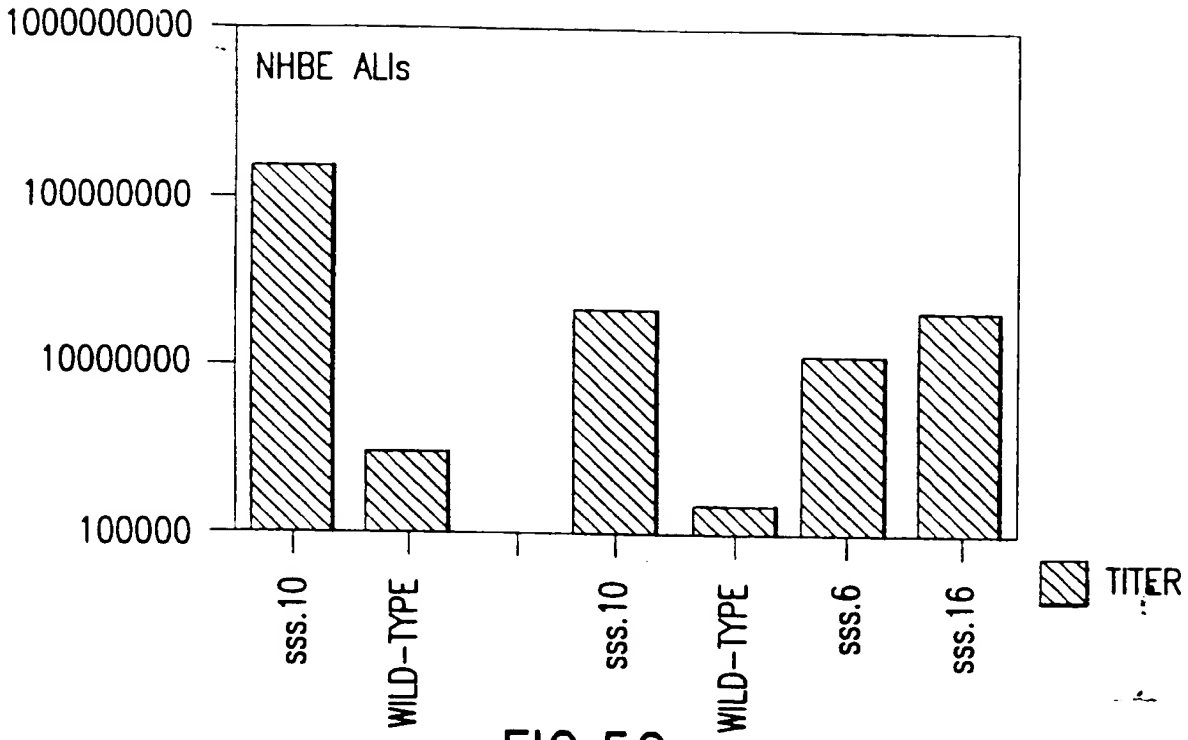

1000000000
100000000
10000000
100000
NHBE ALIs
sss.10
WILD-TYPE
sss.10
WILD-TYPE
sss.6
sss.16
TITER

FIG.5C

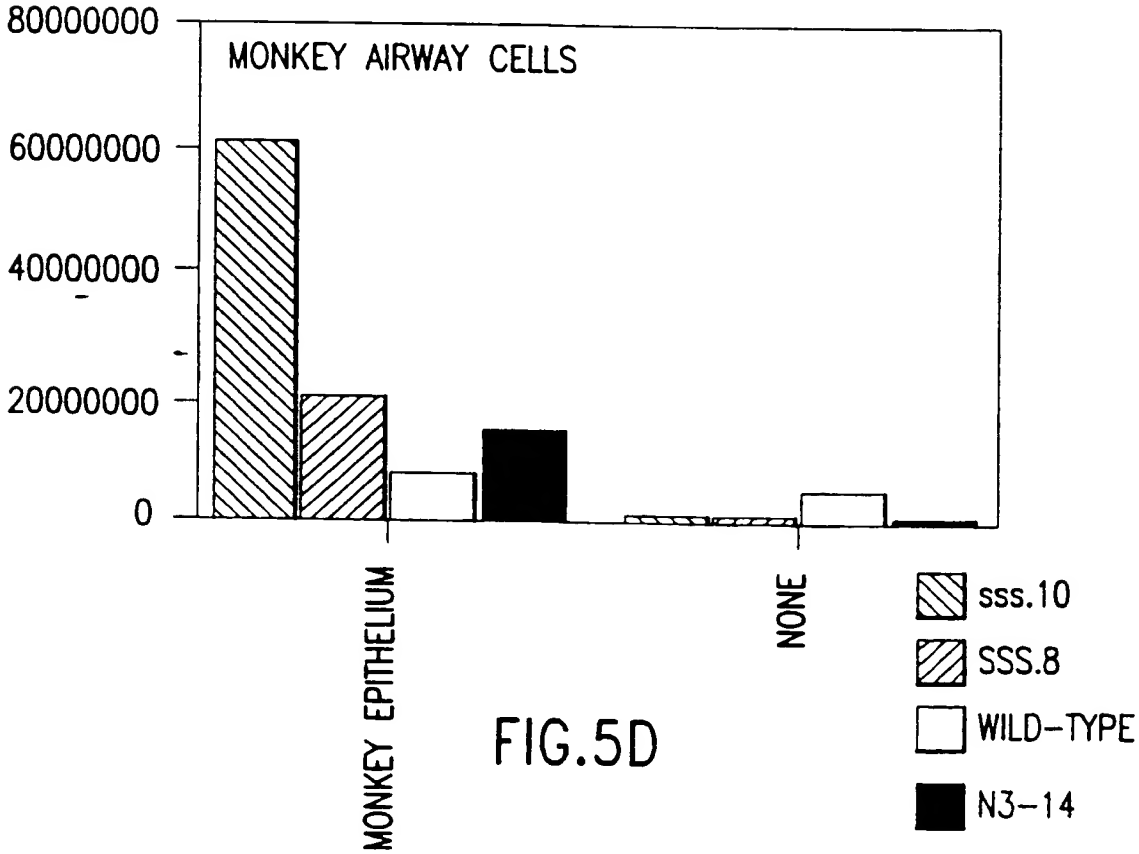

80000000
60000000
40000000
20000000
0
MONKEY AIRWAY CELLS
MONKEY EPITHELIUM
NONE
sss.10
SSS.8
WILD-TYPE
N3-14

FIG.5D

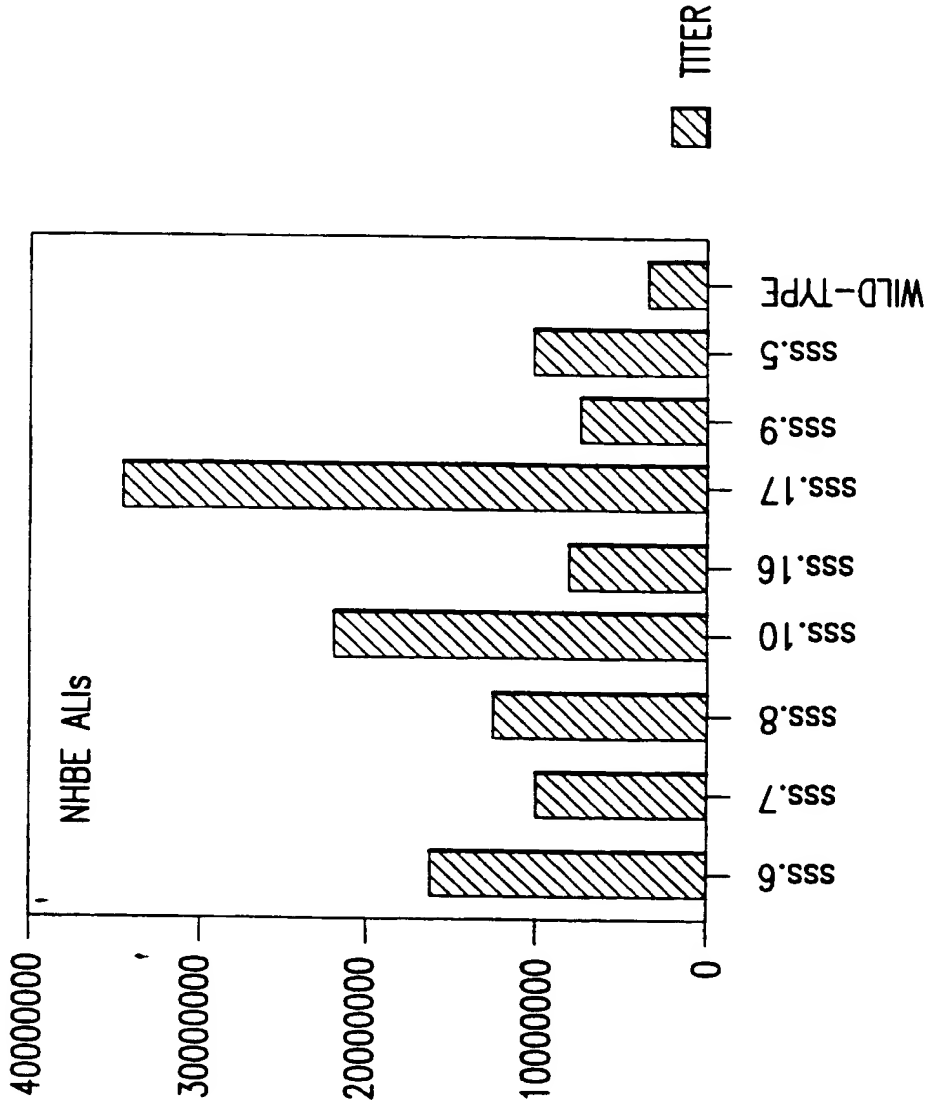
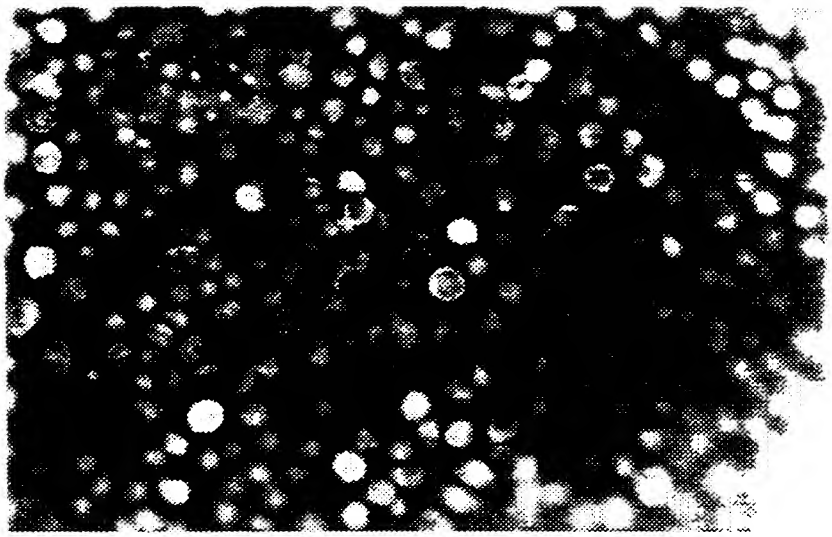

TITER
WILD-TYPE
sss.5
sss.9
sss.17
sss.16
sss.10
sss.8
sss.7
sss.6
NHBE ALIs
40000000
30000000
20000000
10000000
0

FIG.6A



N3 -14

FIG.6B

sss.17

FIG.6C

sss.10

FIG.6D

wild-type

FIG.6E

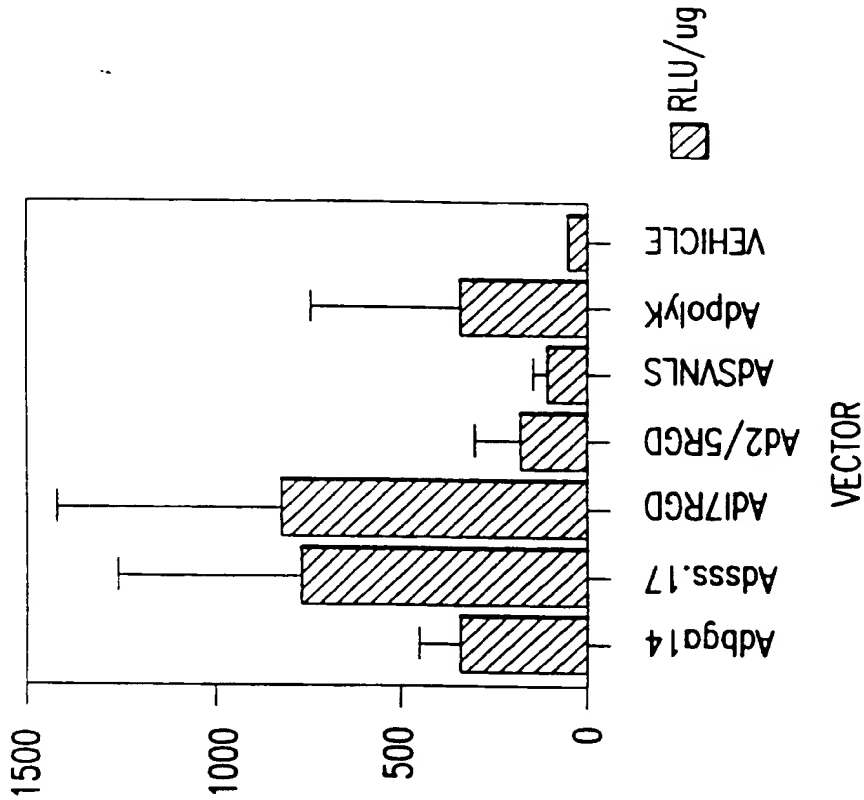

RLU/ug
1500
1000
500
0
Adbga14
Adsss.17
Ad17RGD
Ad2/5RGD
AdSVNLS
Adpolyk
VEHICLE
VECTOR

FIG.8

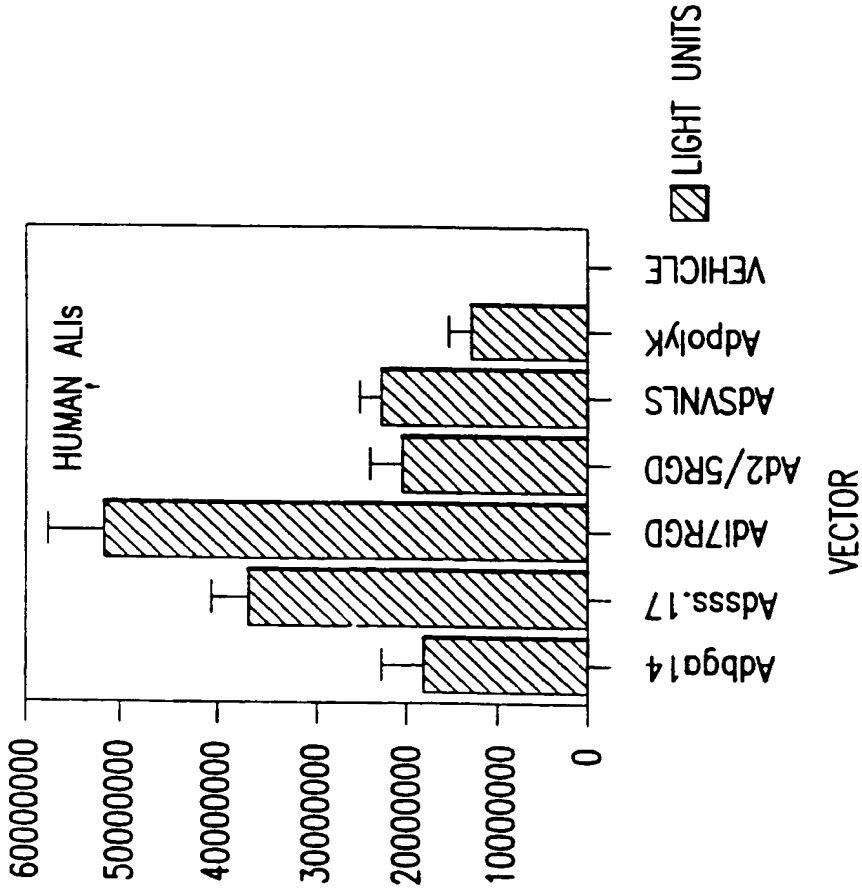

LIGHT UNITS
HUMAN ALIs
60000000
50000000
40000000
30000000
20000000
10000000
0
Adbga14
Adsss.17
Ad17RGD
Ad2/5RGD
AdSVNLS
Adpolyk
VEHICLE
VECTOR

FIG.7

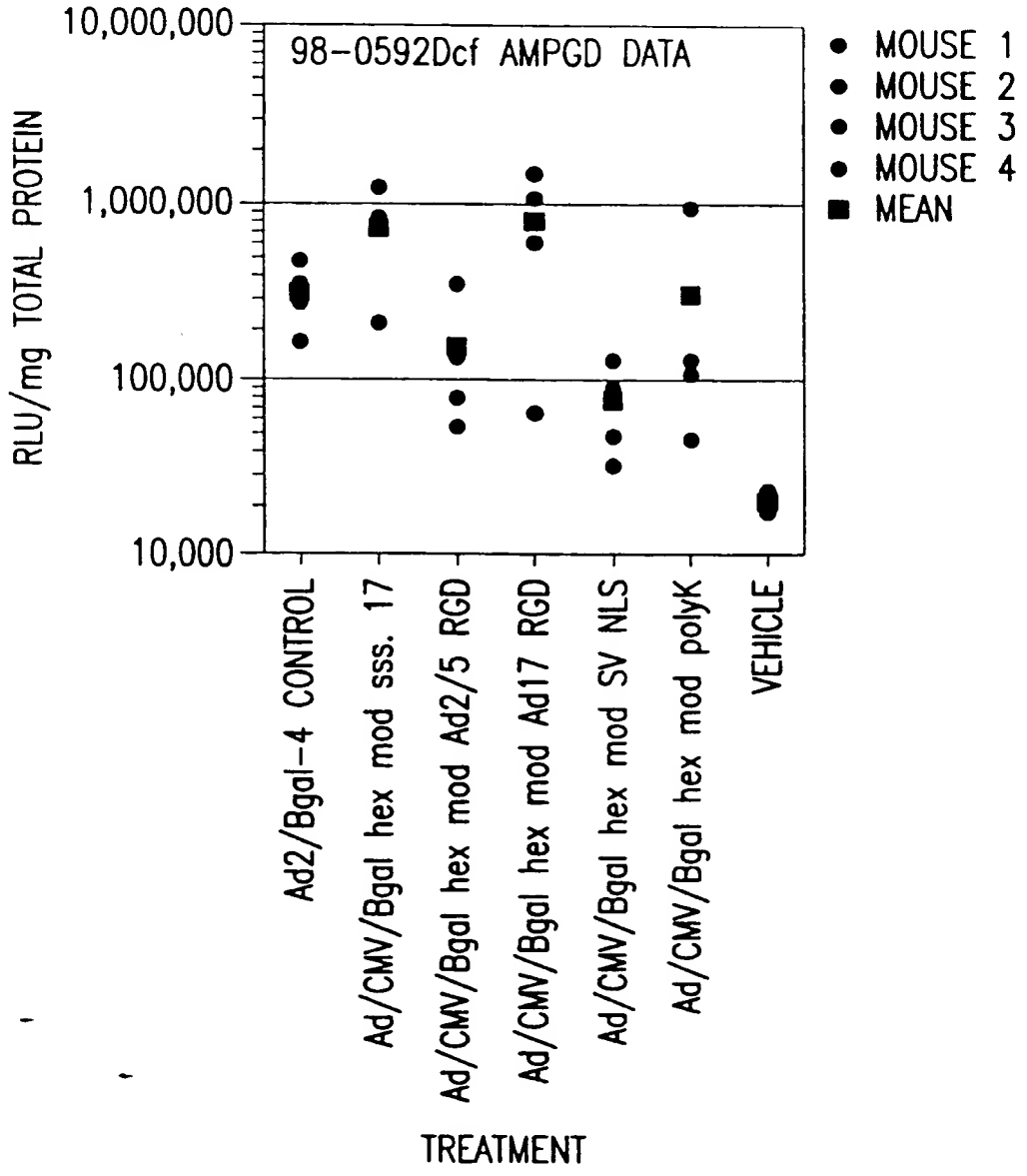

98-0592Dcf AMPGD DATA
10,000,000
1,000,000
100,000
10,000
RLU/mg TOTAL PROTEIN
MOUSE 1
MOUSE 2
MOUSE 3
MOUSE 4
MEAN
Ad2/Bgal-4 CONTROL
Ad/CMV/Bgal hex mod sss. 17
Ad/CMV/Bgal hex mod Ad2/5 RGD
Ad/CMV/Bgal hex mod Ad17 RGD
Ad/CMV/Bgal hex mod SV NLS
Ad/CMV/Bgal hex mod polyK
VEHICLE
TREATMENT

FIG.9

SEQUENCE LISTING

<110> Romanczuk, Helen
Armentano, Donna
O'Riordan, Catherine E.

<120> ADENOVIRAL VECTORS WITH MODIFIED CAPSID PROTEINS

<130> 31366-PCT

<150> 60/071,674
<151> 1998-01-16

<160> 20

<170> FastSEQ for Windows Version 3.0

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<212> PRT
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1 5

<210> 2
<211> 9
<212> PRT
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Gly Pro Ala Arg Gly Asp Ser Ser Val
1 5

<210> 3
<211> 7
<212> PRT
<213> SV40 (Virus)

<400> 3
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1 5

<210> 4
<211> 12
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<213> Artificial sequence

<220>
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<210> 5
<211> 10
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<220>
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<210> 6
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<212> PRT
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<210> 7
<211> 12
<212> PRT
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<220>
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<210> 8
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<210> 10
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1 5 10

<210> 13
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<220>
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Lys Val Val Leu Tyr Ser Glu Asp Val Asn Met
20 25

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20 25

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20 25

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<211> 27
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20 25

<210> 18
<211> 27
<212> PRT
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1 5 10 15
Lys Val Val Leu Tyr Ser Glu Asp Val Asn Met
20 25

<210> 19
<211> 26
<212> PRT
<213> Adenovirus

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1 5 10 15
Gly Val Val Leu Tyr Ser Glu Asp Val Asn
20 25

<210> 20
<211> 12
<212> PRT
<213> Adenovirus

<400> 20
Pro Gly Ser Ala Ser Gly Ser Ala Ser Gly Ser Pro
1 5 10

wt L1	...SNTTSLNDRQGNATKPKVVLYSEDVNM...	SEQ ID NO.14
SSS.17	SNTTSSDQLASPYSHPRVVLYSEDVN	SEQ ID NO.15
Ad 17 RGD	...SNTTSGPARGDSSVKPKVVLYSEDVNM...	SEQ ID NO.16
Ad 2/5 RGD	...SNTTSHAIRGDTFAKPKVVLYSEDVNM...	SEQ ID NO.17
SV40 NLS	...SNTTSLPKKRKVNAPKVVLYSEDVNM...	SEQ ID NO.18
polyK	SNTTSKGKGKGKGKGKGVVLYSEDVN	SEQ ID NO.19

FIG. 1A

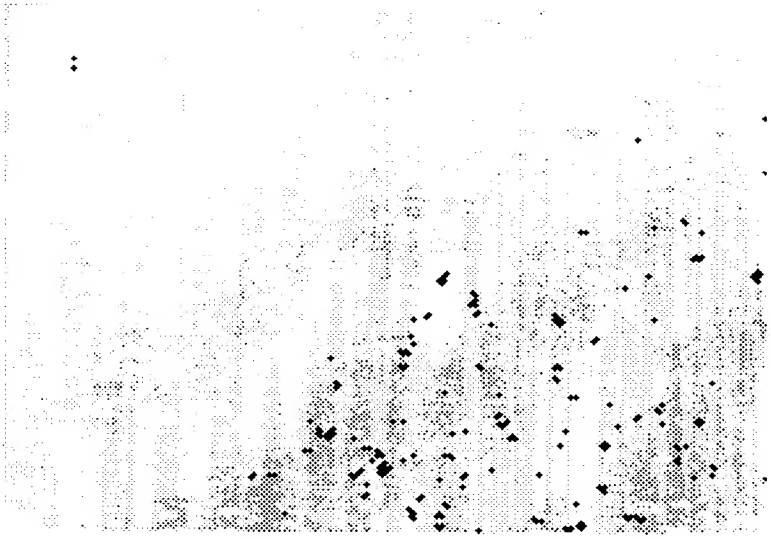


FIG. 1B

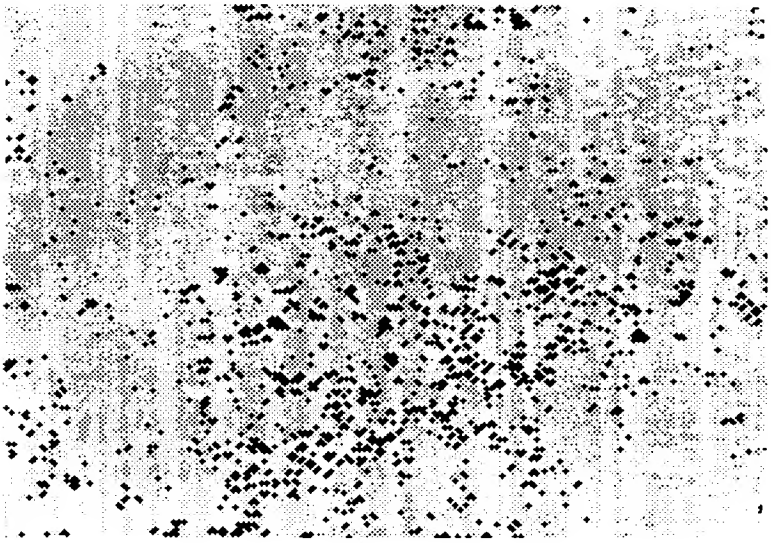


FIG. 1C

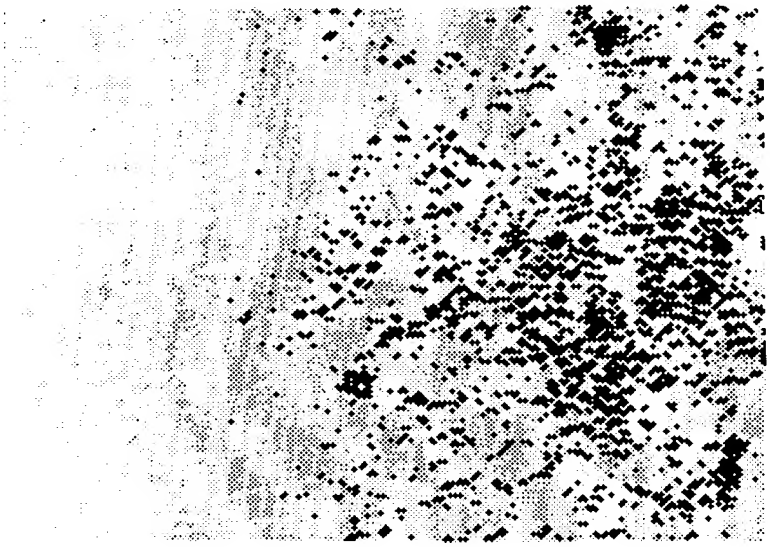


FIG. 1D

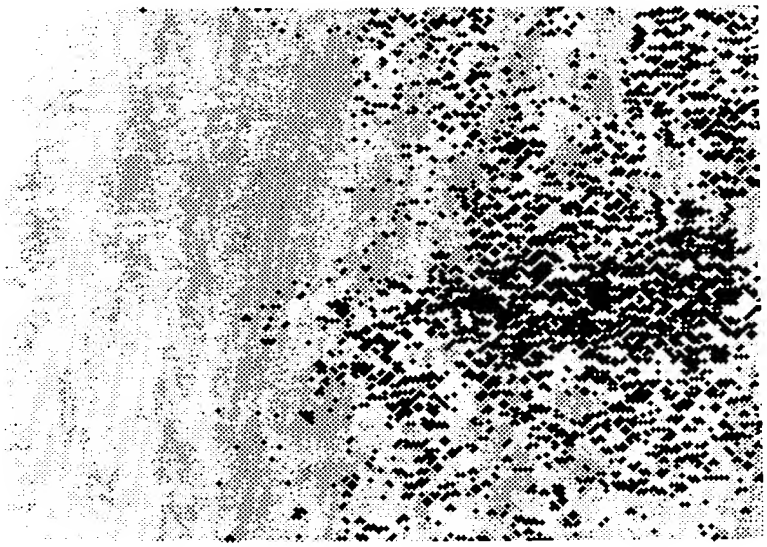


FIG. 1E

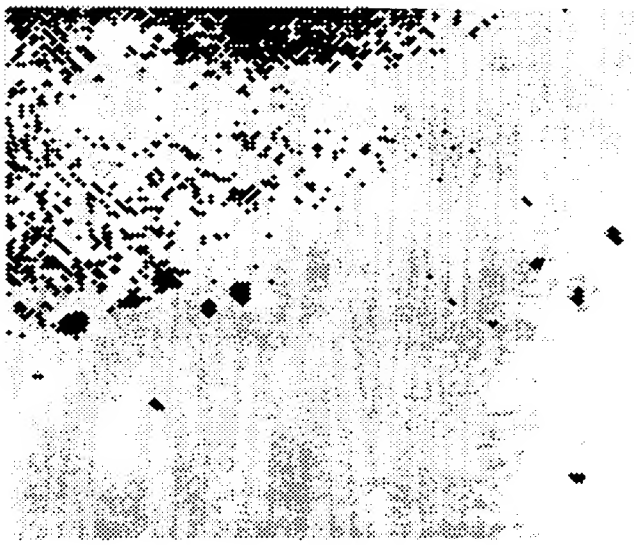


FIG. 1F

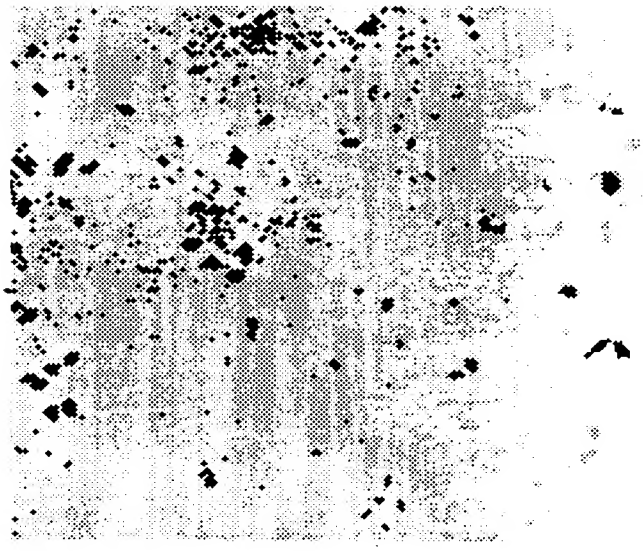


FIG. 1G

FIG. 1H

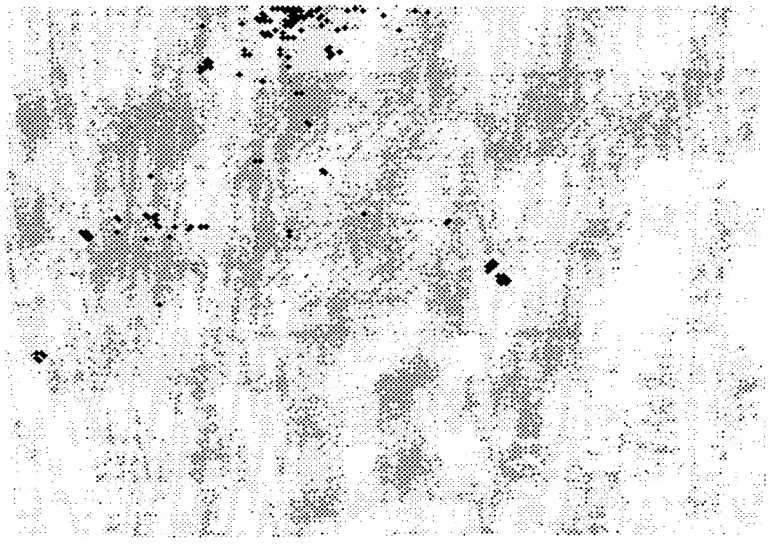


FIG. 1I

RLU
400000
300000
200000
100000
0
Ad2/bgal-4
hex.mod.2/5RGD
hex.mod.17RGD
hex.mod.SV40NLS
VIRAL VECTOR

FIG.1J

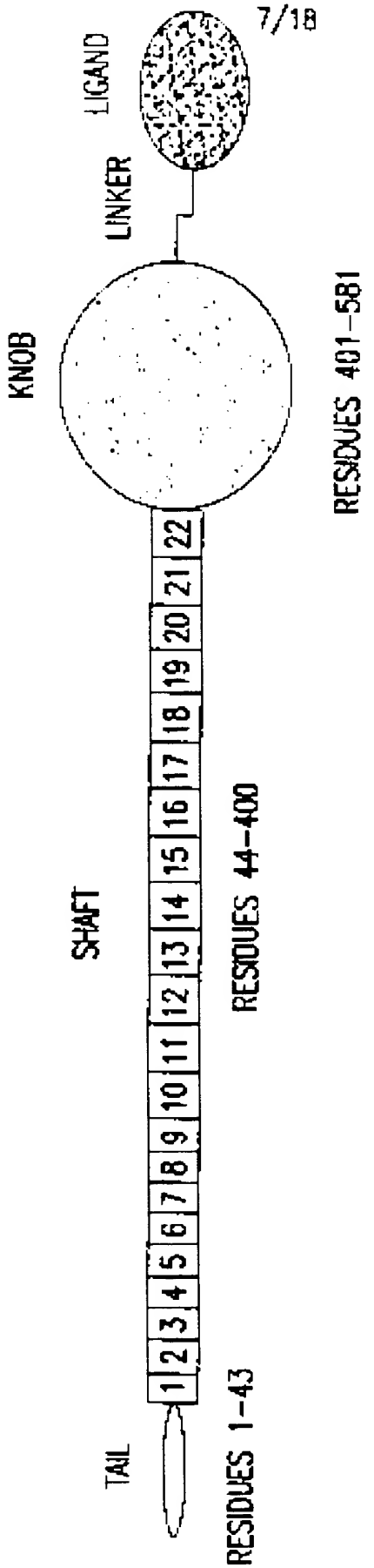

LIGAND
LINKER
KNOB
RESIDUES 401-581
SHAFT
RESIDUES 44-400
TAIL
RESIDUES 1-43
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

FIG.2A

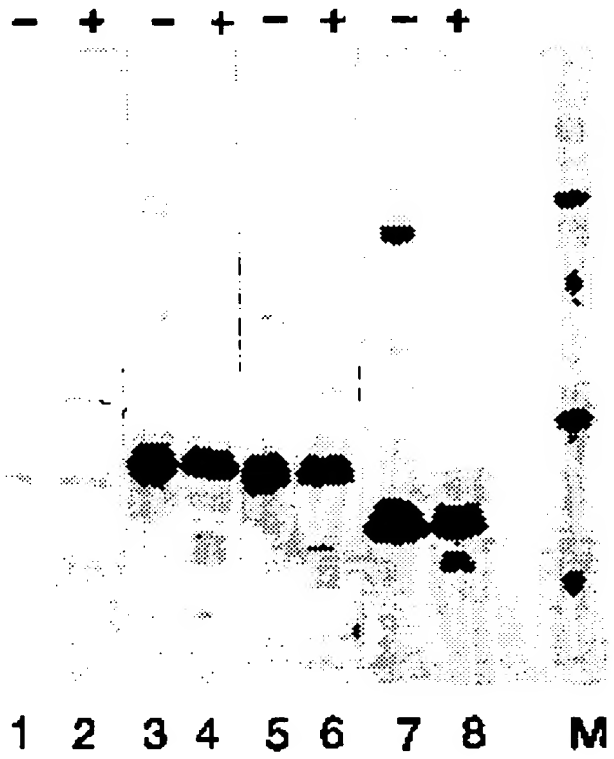


FIG.2B

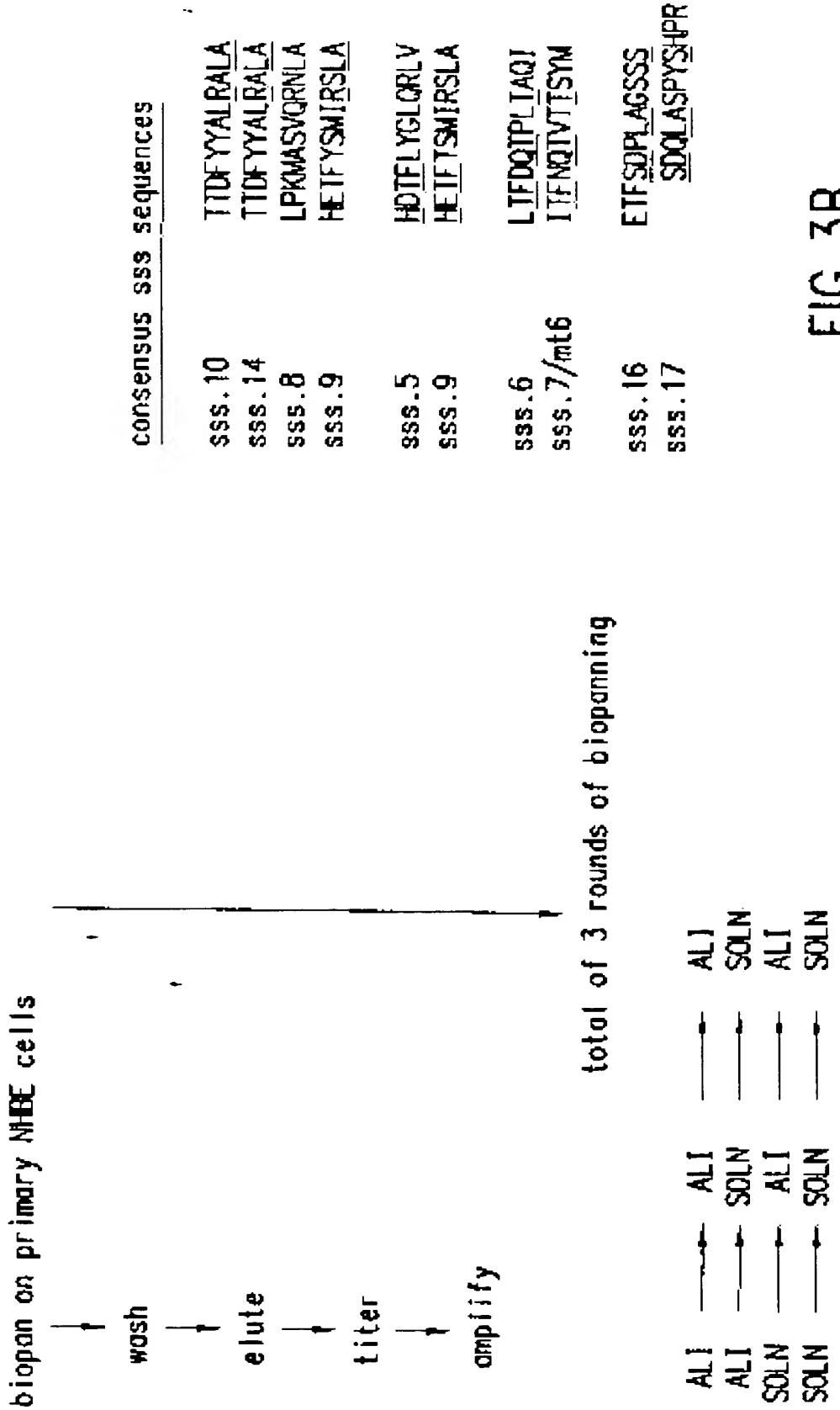


FIG. 3A

consensus sss sequences

sss.10	TTDFYYALRALA
sss.14	TTDFYYALRALA
sss.8	LPKMASVQRNLA
sss.9	HETFYSWIRSLA
sss.5	HDTFLYGLQRLV
sss.9	HETFTSWIRSLA
sss.6	LTFDQTPLIAQI
sss.7/mt6	ITFNQTVTTSYW
sss.16	ETFSDPLAGSSS
sss.17	SDQLASPYSHPR

FIG. 3B

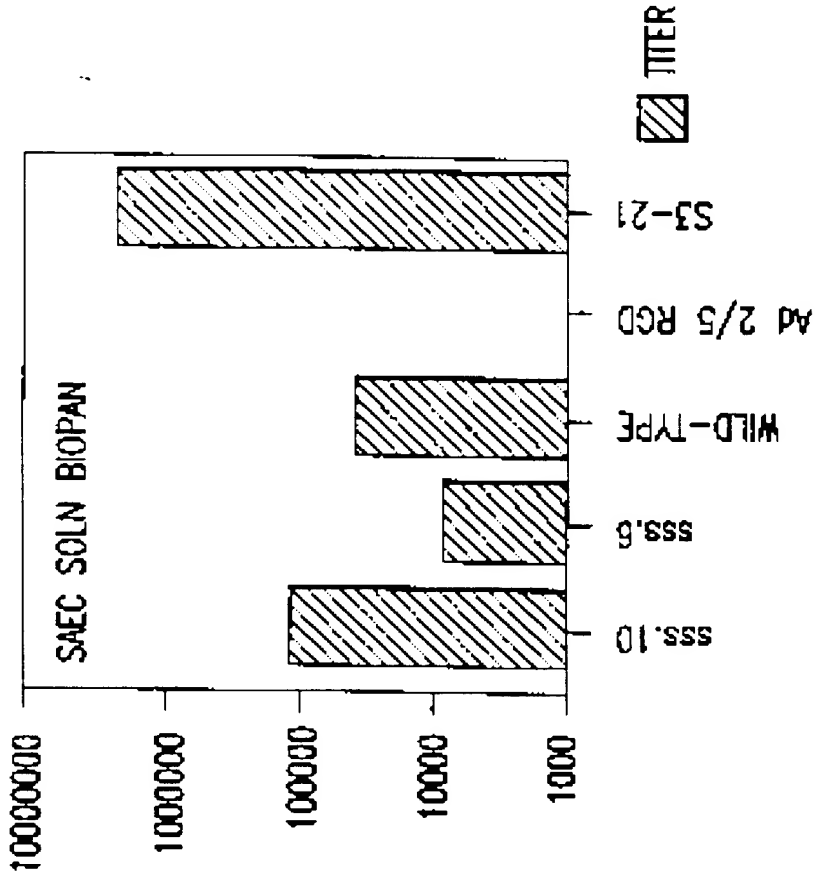

TITER
SAEC SOLN BIOPAN
10000000
1000000
100000
10000
1000
sss.10
sss.6
WILD-TYPE
Ad 2/5 RGD
S3-21

FIG.4B

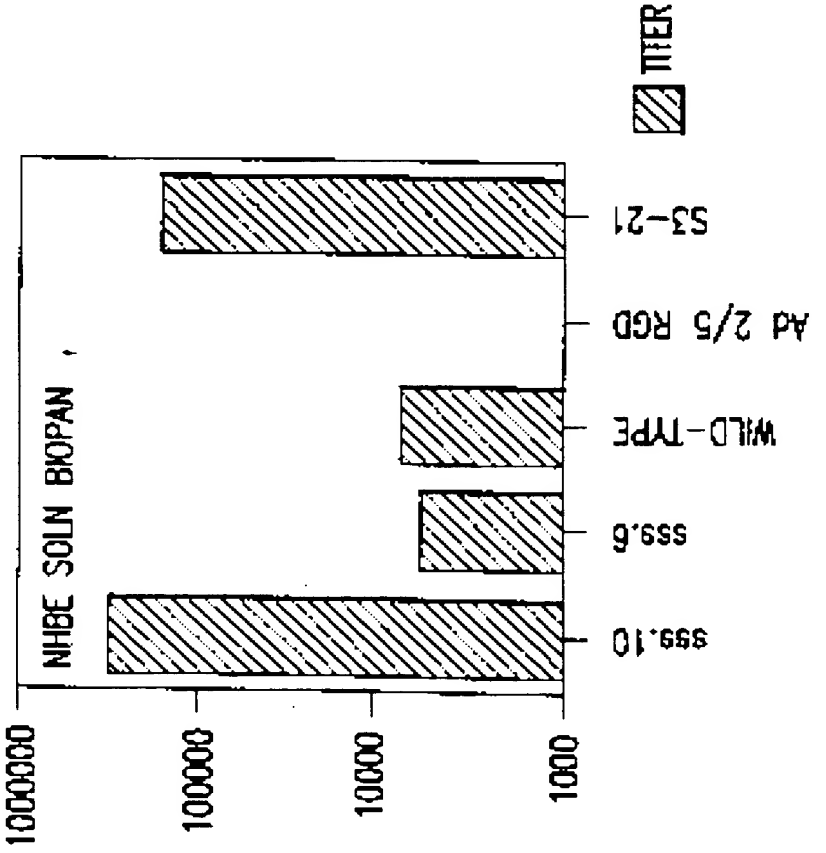

TITER
NHBE SOLN BIOPAN
1000000
100000
10000
1000
sss.10
sss.6
WILD-TYPE
Ad 2/5 RGD
S3-21

FIG.4A

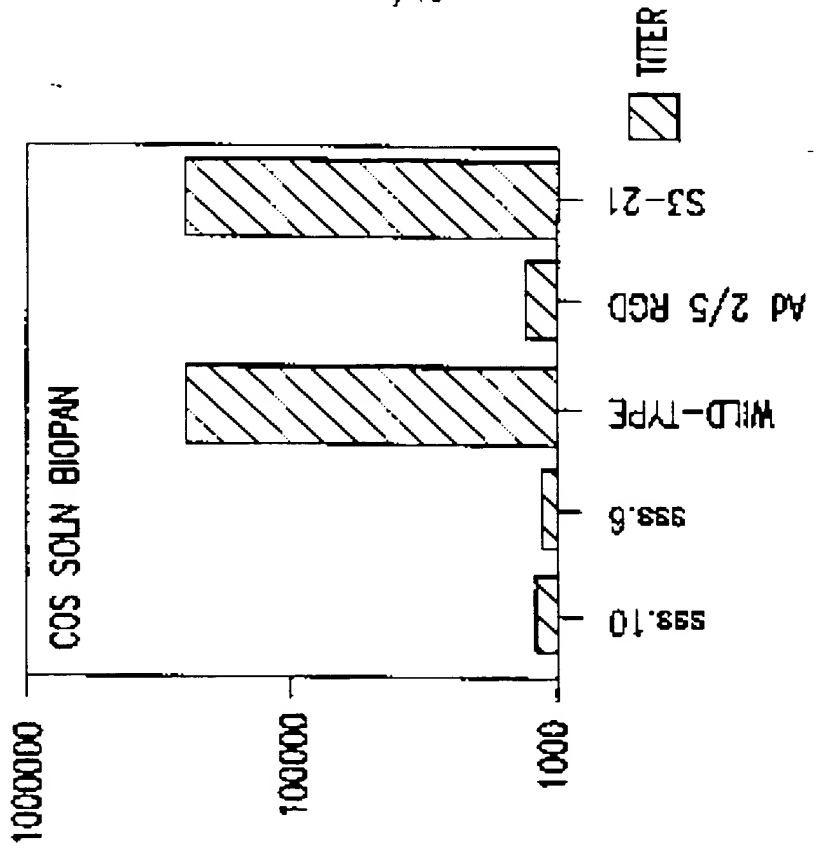
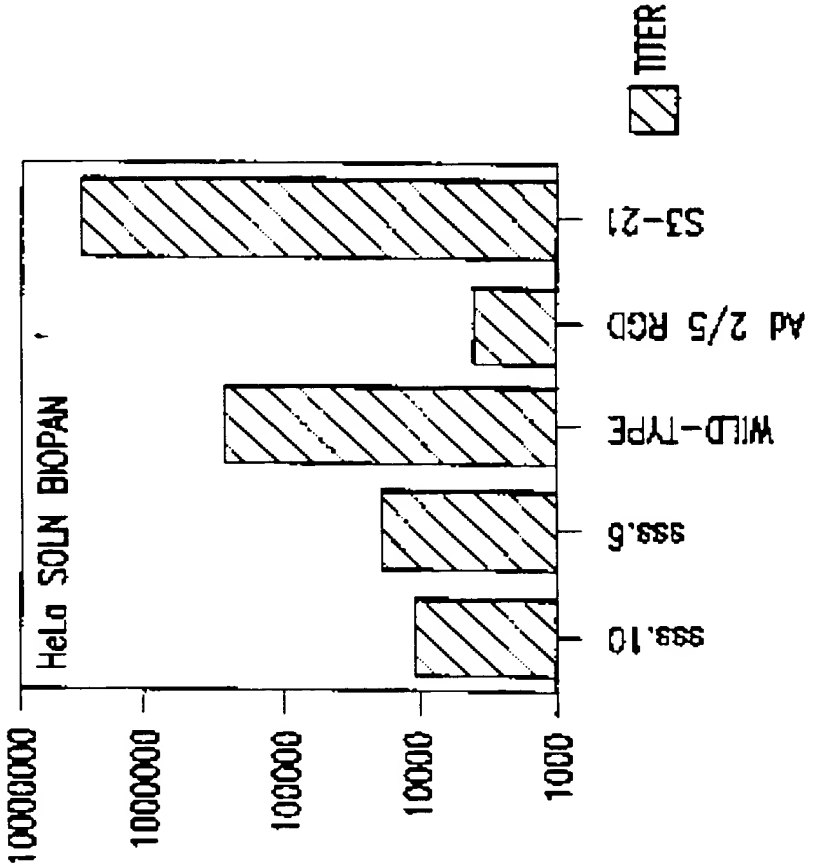

TITER
COS SOLN BIOPAN
1000000
100000
1000
sss.10
sss.6
WILD-TYPE
Ad 2/5 RGD
S3-21

FIG.4D


TITER
HeLa SOLN BIOPAN
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1000000
100000
10000
1000
sss.10
sss.6
WILD-TYPE
Ad 2/5 RGD
S3-21

FIG.4C

FIG.5B

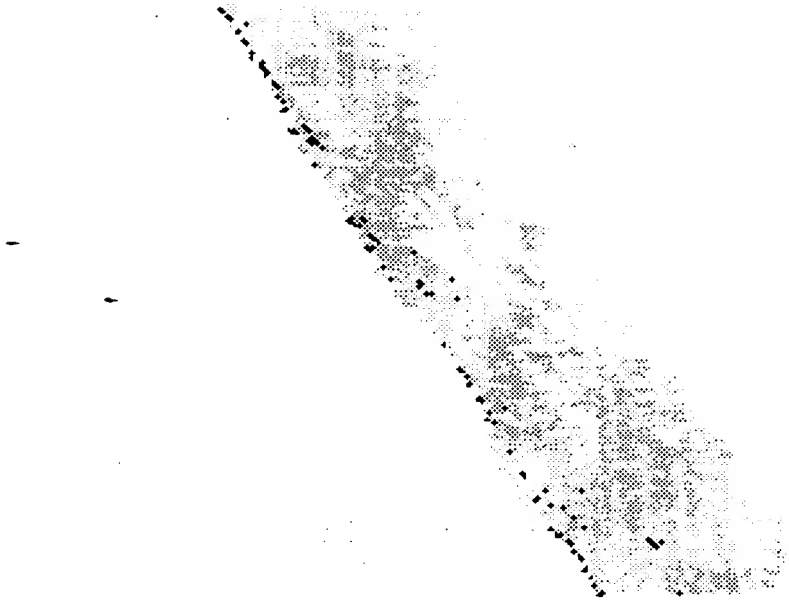


FIG.5A

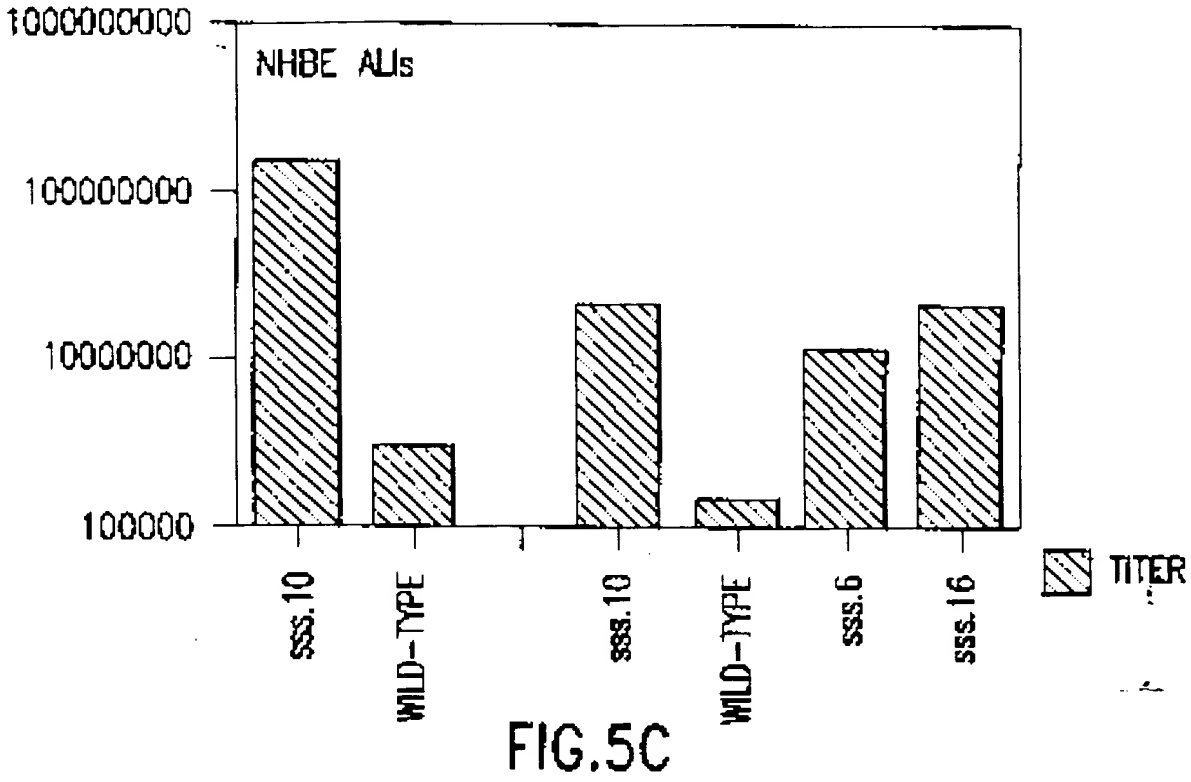

1000000000
100000000
10000000
100000
NHBE ALIs
SSS.10
WILD-TYPE
SSS.10
WILD-TYPE
SSS.6
SSS.16
TITER

FIG.5C

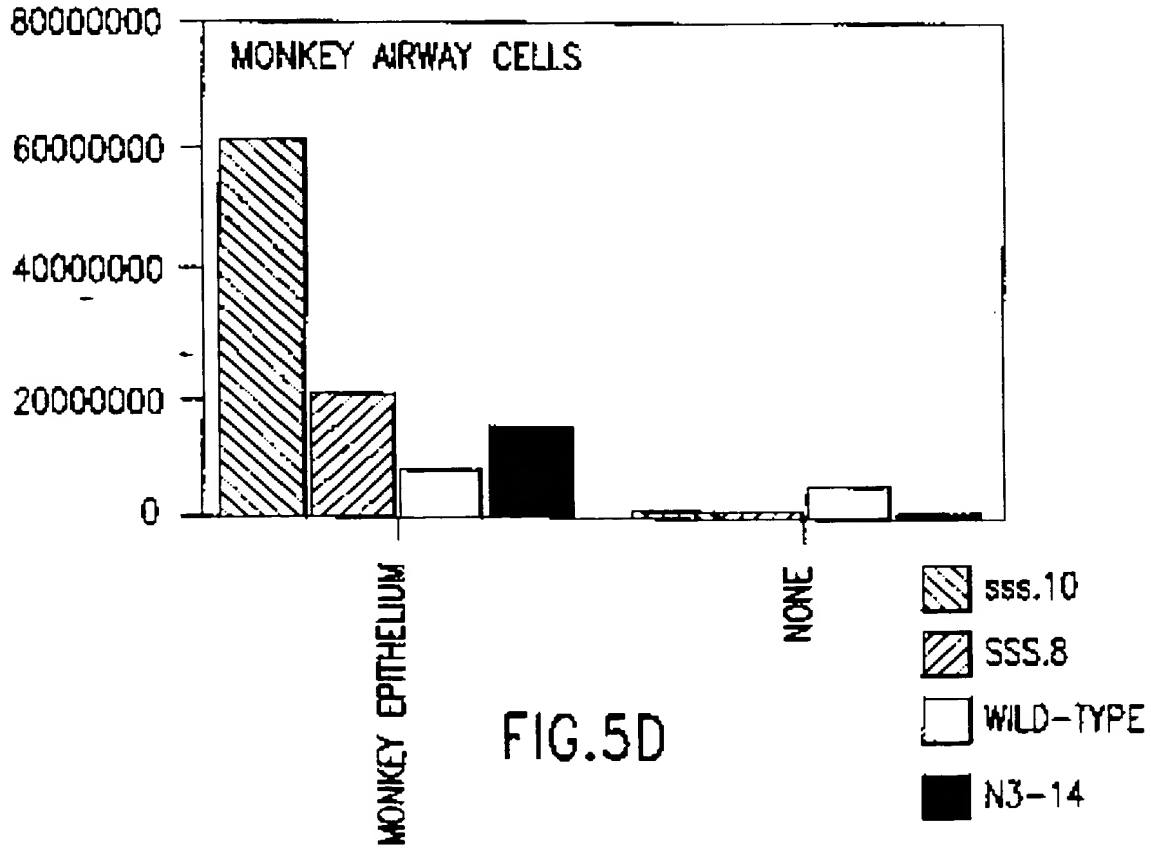

80000000
60000000
40000000
20000000
0
MONKEY AIRWAY CELLS
MONKEY EPITHELIUM
NONE
SSS.10
SSS.8
WILD-TYPE
N3-14

FIG.5D

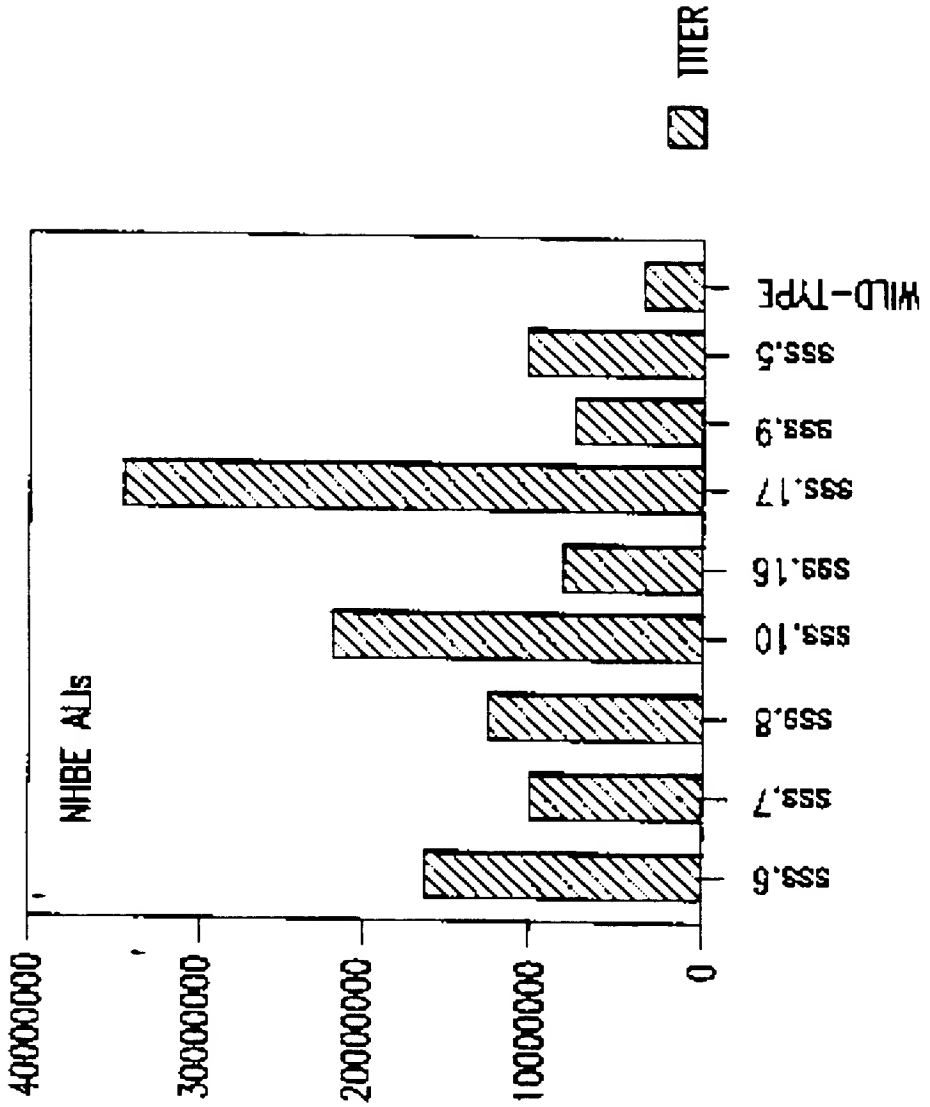
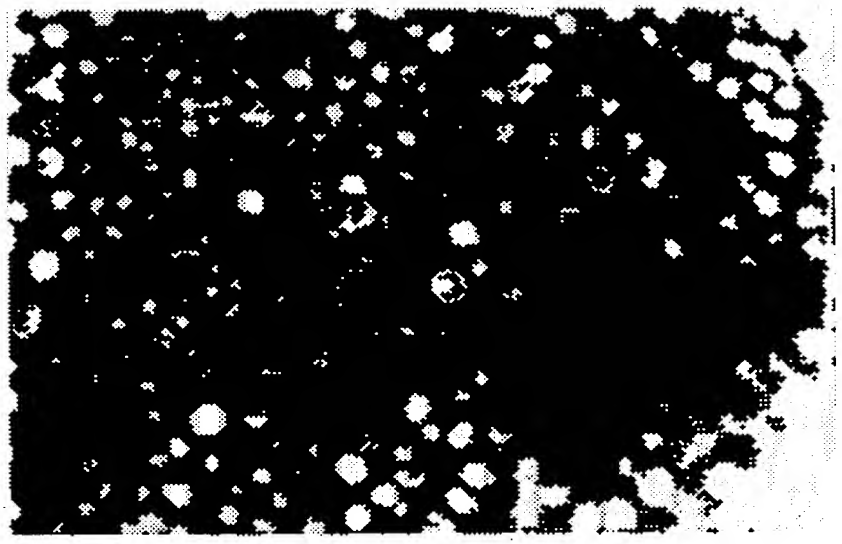

TITER
WILD-TYPE
sss.5
sss.9
sss.17
sss.16
sss.10
sss.8
sss.7
sss.6
NHBE ALIs
40000000
30000000
20000000
10000000
0

FIG.6A



N3 -14

FIG.6B

sss.17

FIG.6C

888.10

FIG.6D

wild-type

FIG.6E

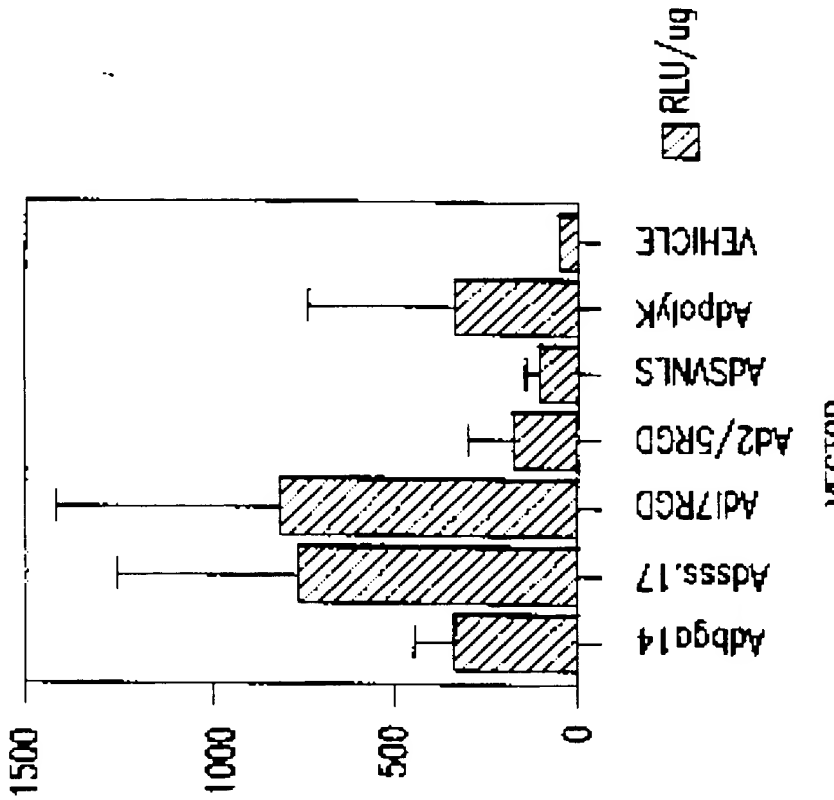
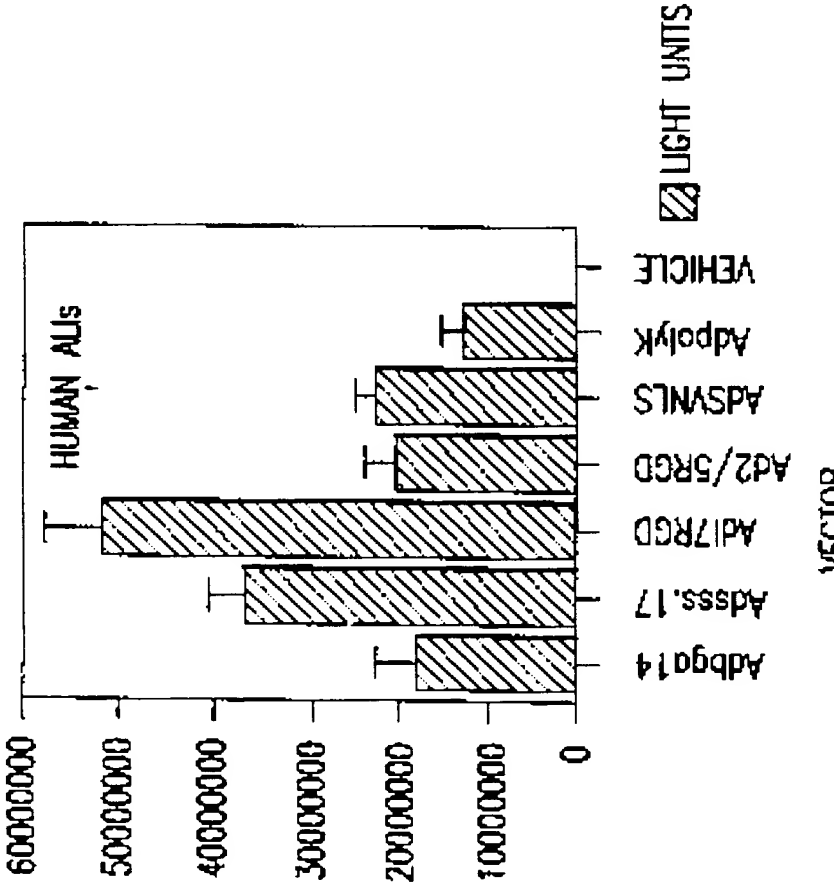

RLU/ug
VECTOR
Adbga14
Adsss.17
Ad17RGD
Ad2/5RGD
AdSVNLS
Adpolyk
VEHICLE
0
500
1000
1500

FIG.8


LIGHT UNITS
HUMAN ALIs
VECTOR
Adbga14
Adsss.17
Ad17RGD
Ad2/5RGD
AdSVNLS
Adpolyk
VEHICLE
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10000000
20000000
30000000
40000000
50000000
60000000

FIG.7

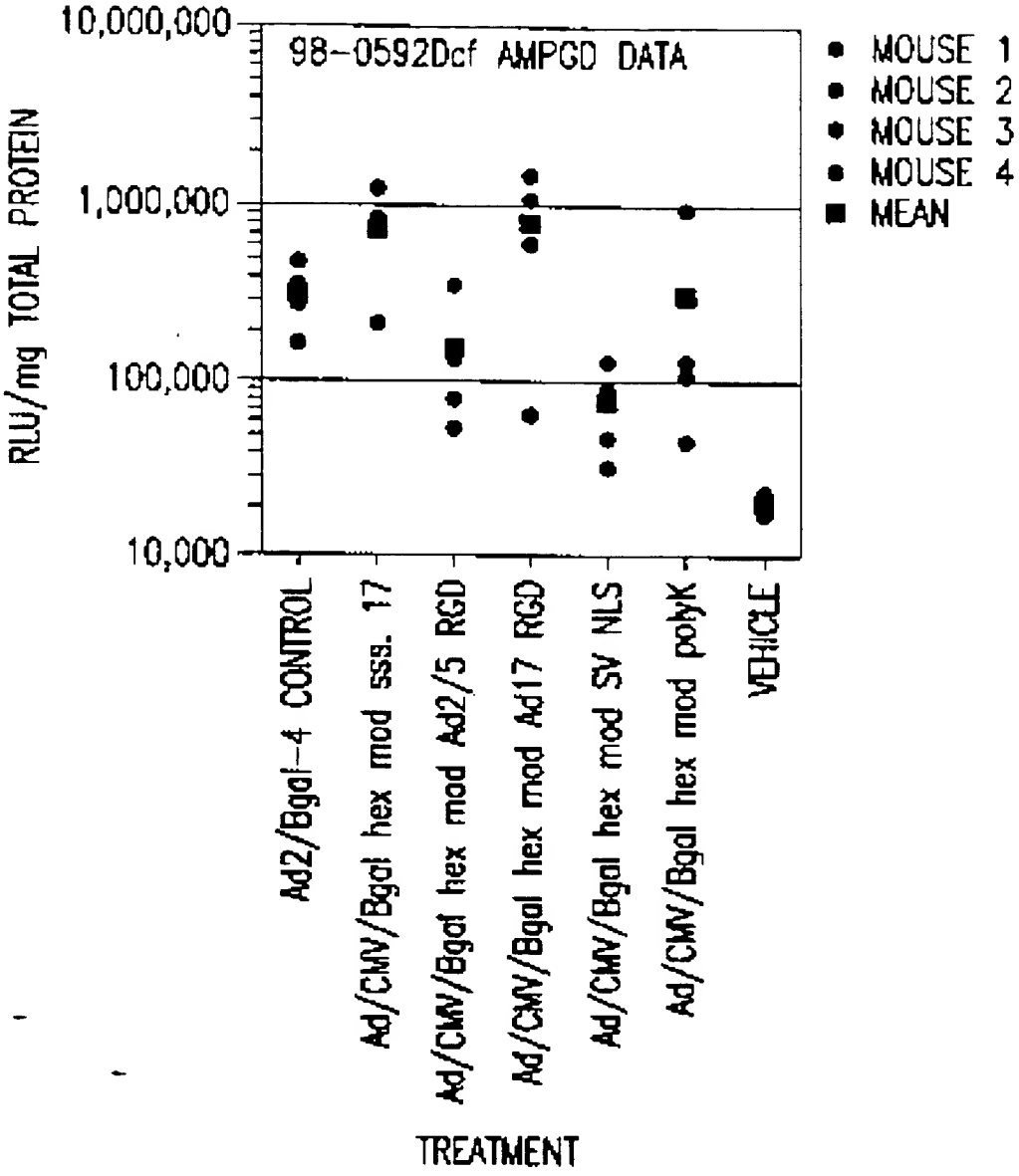
98-0592Dcf AMPGD DATA
RLU/mg TOTAL PROTEIN
10,000,000
1,000,000
100,000
10,000
MOUSE 1
MOUSE 2
MOUSE 3
MOUSE 4
MEAN
Ad2/Bgal-4 CONTROL
Ad/CMV/Bgal hex mod sss. 17
Ad/CMV/Bgal hex mod Ad2/5 RGD
Ad/CMV/Bgal hex mod Ad17 RGD
Ad/CMV/Bgal hex mod SV NLS
Ad/CMV/Bgal hex mod polyK
VEHICLE
TREATMENT

FIG.9

SEQUENCE LISTING

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Armentano, Donna
O'Riordan, Catherine R.

<120> ADENOVIRAL VECTORS WITH MODIFIED CAPSID PROTEINS

<130> 31366-PCT

<150> 60/071,674
<151> 1998-01-16

<160> 20

<170> FastSEQ for Windows Version 3.0

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<220>
<223> Randomly synthesized

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<212> PRT
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Lys Val Val Leu Tyr Ser Glu Asp Val Asn Met
20 25

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<212> PRT
<213> Adenovirus

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Arg Val Val Leu Tyr Ser Glu Asp Val Asn
20 25

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<212> PRT
<213> Adenovirus

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Lys Val Val Leu Tyr Ser Glu Asp Val Asn Met
20 25

<210> 17
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<212> PRT
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20 25

<210> 18
<211> 27
<212> PRT
<213> Adenovirus

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Lys Val Val Leu Tyr Ser Glu Asp Val Asn Met
20 25

<210> 19
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1 5 10 15
Gly Val Val Leu Tyr Ser Glu Asp Val Asn
20 25

<210> 20
<211> 12
<212> PRT
<213> Adenovirus

<400> 20
Pro Gly Ser Ala Ser Gly Ser Ala Ser Gly Ser Pro
1 5 10

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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Filed on 16 January 1998 (16.01.98)

(71) Applicant *(for all designated States except US)*: GENZYME CORPORATION [US/US]; One Mountain Road, Framingham, MA 01701 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: ROMANCZUK, Helen [US/US]; 3101 Windsor Ridge Drive, Westboro, MA 01581 (US). ARMENTANO, Donna [US/US]; 352 Brighton Street, Belmont, MA 02178 (US). O'RIORDAN, Catherine, R. [IE/US]; 70 Revers Street, Boston, MA 02114 (US).

(74) Agent: SEIDE, Rochelle, K.; Baker & Botts, LLP, 30 Rockefeller Plaza, New York, NY 10112-0228 (US).

(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

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(54) Title: ADENOVIRAL VECTORS WITH MODIFIED CAPSID PROTEINS

(57) Abstract

The present invention is directed to adenoviral vectors having modified capsid proteins which comprise heterologous ligands that improve and/or alter the infectious capability of the vector. Such ligands are capable of binding to target cells, and their inclusion into adenoviral vectors facilitates the binding and infectious properties of the vectors. In a preferred embodiment, the ligands are peptides, and the target cells are epithelial cells. The invention is also directed to novel heterologous ligands, to ligand–receptor complexes, and to compositions comprising the adenoviral vectors of the invention. Additional aspects of the invention include methods to use the adenoviral vectors of the invention to deliver transgenes to target cells.

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INTERNATIONAL SEARCH REPORT

Internatic Application No
PCT/US 99/00913

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/34 C12N15/86 C07K14/075 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	WO 97 20051 A (GENVEC INC ;WICKHAM THOMAS J (US); KOVESDI IMRE (US); BROUGH DOUGL) 5 June 1997 see page 11, line 21 - line 32; examples 2,15	1-3, 17-19, 43-46
X	WO 95 05201 A (GENETIC THERAPY INC ;MCCLELLAND ALAN (US); STEVENSON SUSAN C (US)) 23 February 1995 see examples 1,2,4	1,2,17, 18,43,44
X	WO 95 26412 A (UAB RESEARCH FOUNDATION) 5 October 1995 see page 14, line 27 - page 15, line 10	1,2,17, 18,43,44
X	WO 97 20575 A (UNIV ALABAMA AT BIRMINGHAM RES) 12 June 1997 see examples 2-10	1,2,17, 18,43,44

-/--

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Date of the actual completion of the international search	Date of mailing of the international search report
3 June 1999	24 09. 1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	CUPIDO, M

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INTERNATIONAL SEARCH REPORT

Intematic Application No
PCT/US 99/00913

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
P,X	WO 98 22609 A (ARMENTANO DONNA E ;GREGORY RICHARD J (US); GENZYME CORP (US); SMIT) 28 May 1998 see figure 5A; example 6 ---	1,2,5,7, 9,11,17, 18,21, 23,29, 31,32, 35,37, 43-45, 48,50
X,P	FR 2 761 688 A (TRANSGENE SA) 9 October 1998 see examples 1-4 -----	1-3, 17-19, 43-46

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US 99/00913

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1 [X] Claims Nos.
because they relate to subject matter not required to be searched by this Authority, namely

Remark: Although claims 43-51, insofar they refer to in vivo methods, are directed to a method of treatment of the human or animal body, the search has been carried out and based on the alleged effects of the adenoviral vector.

2 [ ] Claims Nos.
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3 [ ] Claims Nos.
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-4, 9-28, 31-51

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-4, 9-28, 31-51

Adenoviral capsid proteins comprising a heterologous ligand facilitating binding of adenovirus to a target cell, adenoviral vectors comprising them and their use to infect target cells.

2. Claims: 5-8, 29 and 30

Heterologous ligands, oligonucleotides encoding them and complexes thereof with a cellular receptor.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/00913

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